



## REVIEW

# Circulating endothelial progenitor cells and chronic pulmonary diseases

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**ABSTRACT:** Circulating endothelial progenitor cells (EPCs) are bone marrow-derived cells that contribute to vascular healing and remodelling under physiological and pathological conditions. Although controversies exist regarding the definition and origin of EPCs, it has been widely demonstrated that they are involved in several diseases and that they have therapeutic implications.

Chronic obstructive pulmonary disease (COPD) is characterised by airflow limitation that is not fully reversible, associated with abnormalities of airways (bronchitis) and parenchyma (emphysema), reduced exercise tolerance and systemic inflammation.

Growing evidence has also suggested that endothelial dysfunction may play a role in COPD. Although it is not clear whether endothelial dysfunction represents a cause or a consequence of COPD, several studies have highlighted the importance of EPCs in this disease, suggesting that the bone marrow could be a novel target of COPD.

The present review summarises the role of EPCs in pulmonary diseases, with particular emphasis on COPD. The aim is to improve understanding as to the possible role of EPCs in COPD pathophysiology. This may help in the identification of novel diagnostic and therapeutic tools in COPD.

**KEYWORDS:** Chronic obstructive pulmonary disease, endothelium

In 1997, ASAHARA *et al.* [1] first described the differentiation of adult haemopoietic progenitor cells into an endothelial phenotype. In 1998, SHI *et al.* [2] showed that genetically tagged transplanted bone marrow cells were covering implanted grafts. These pioneering studies suggested the presence of circulating angioblasts in the adult peripheral blood. Since the late 1990s, circulating haemopoietic and endothelial progenitor cells (EPCs) have been studied in order to characterise and understand their role in the pathophysiology of various diseases. Accumulating evidence has shown that circulating EPCs contribute to vascular healing and remodelling under physiological and pathological conditions. Despite some controversies still existing with respect to the identification and origin of these progenitor cells, increasing evidence suggests that these bone marrow-derived cells play an important role in diseases, such as cardiovascular and cerebrovascular, endocrinological, haematological and connective tissue disorders [3]. Plasma levels of these circulating EPCs have been seen to correlate with disease severity and risk factors [4]. In contrast to the

conventional assumption that damaged organs are repaired only by migration and proliferation of adjacent cells, increasing evidence suggests that ectopic progenitor cells are mobilised into the systemic circulation and recruited to the site of tissue regeneration. Furthermore, EPCs also seem to have therapeutic value in coronary artery diseases, increasing neovascularisation of tissue following ischaemia [5–7] and contributing to re-endothelialisation after endothelial injury [8, 9].

The aims of the present review are, first, to provide a state-of-the-art overview of circulating EPCs and pulmonary diseases, with particular emphasis on chronic obstructive pulmonary disease (COPD) and emphysema, and then to present recent findings in the field, which may have implications for diagnostic tools and novel therapeutic strategies in chronic lung diseases.

## CIRCULATING EPCS

### Definition

In 1997, ASAHARA *et al.* [1] showed that purified CD34+ haemopoietic EPCs from human peripheral blood could differentiate into endothelial cells (ECs) *in vitro* [1]. These so-called endothelial

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progenitor cells (EPCs) showed expression of various EC markers and migrated to sites of active angiogenesis in several animal models. This behaviour suggested that they might be useful for targeted delivery of therapeutic agents that stimulate or inhibit angiogenesis. Until that moment, production of new blood vessels in adults had been thought to result exclusively from the proliferation, migration and remodelling of fully differentiated ECs derived from pre-existing blood vessels [10, 11]. According to this initial discovery, EPCs were defined as cells positive for both haemopoietic stem cell markers, such as CD34, and an endothelial marker protein, such as kinase insert domain receptor (also known as vascular endothelial growth factor (VEGF) receptor (VEGFR) 2). Since CD34 was not found exclusively on haemopoietic stem cells, but also on mature ECs, several authors used the more immature haemopoietic stem cell marker CD133 (or AC133) to define EPCs [12, 13]. Principle amongst the defining criteria of these endothelioid cells are the presence/absence of the cell surface glycoproteins CD34 (a marker of haematopoietic progenitor cells) [14, 15], CD31, CD146/P1H12 (also frequently used to define circulating ECs), AC133 and fetal liver kinase-1 (another VEGFR), *Bandeirara simplicifolia* lectin 1 binding, the uptake of acetylated low-density lipoprotein-cholesterol, the presence of von Willebrand factor and *in vitro* morphology [2, 16, 17]. Despite this definition, controversies still exist regarding the identification and origin of the EPCs isolated from peripheral blood mononuclear cells. As recently reviewed by URBICH and DIMMELER [18], several sources of ECs may exist: 1) the rare haemopoietic stem cells; 2) myeloid cells which may differentiate *in vitro* to ECs; 3) other circulating progenitor cells, such as adult bone marrow-derived stem/progenitor cells (*e.g.* side-population cells and multipotent adult progenitor cells) [19, 20]; 4) circulating mature ECs [21]; 5) cells not derived from bone marrow, which have been seen to replace ECs in grafts [22]; and 6) tissue-resident stem cells [23].

In 2011, more than ten years after the first description of circulating EPCs, more specific phenotypes are still needed. Hopefully, more surface markers or better profiling of distinct cell populations will soon help to define those cells.

### Role in diseases

Despite the difficulties in defining and characterising circulating ECs, increasing evidence suggests that bone marrow-derived cells play an important role in diseases, such as cardiovascular and cerebrovascular, endocrinological, haematological and connective tissue disorders [3]. Circulating EPCs are decreased in number and show decreased function in

patients with chronic heart failure [24], stroke [25] insulin-dependent [26] and non-insulin-dependent diabetes [27], rheumatoid arthritis [28] and chronic renal failure [29–31]. Acute ischaemia is known to mobilise EPCs, as recently reported in patients with acute myocardial infarction [32–34] and unstable angina [35]. These data suggest that EPCs are physiologically important in maintaining vascular integrity. Interestingly, EPC numbers are decreased in chronic diseases characterised by increased cardiovascular risk, perhaps due to progressive exhaustion of repair capabilities. It is also known that circulating EPC numbers decrease as the number of cardiovascular risk factors increase; patients with coronary artery disease have a lower number of EPCs than healthy controls, [36–39]. These authors also reported that numbers of EPCs correlated inversely with the number of risk factors. Similarly, in males free of a history of clear atherosclerosis, HILL *et al.* [40] reported an inverse correlation between EPC numbers and the Framingham risk factor score, and between EPC numbers and macrovascular function, as assessed by flow-mediated dilatation.

In cardiovascular diseases, the finding that bone marrow-derived cells can migrate to sites of ischaemia and express endothelial surface markers has introduced the challenge of a role for EPCs for therapeutic use. These cells have been shown to actively promote vascular repair, mainly through two mechanisms: neovascularisation, and endothelial regeneration. Several experimental and clinical studies have been performed with different improvement models, demonstrating the possible therapeutic importance of EPCs [6, 7, 9, 41–44]. Statins [36, 45] and angiotensin II receptor antagonists [46] have been seen to partly restore the decreased number of EPCs. Autologous EPCs have been used to improve vascularisation of myocardium or lower limbs in patients with acute myocardial infarction [7] and animals with experimentally induced arterial lesions [47]. In both cases, vascularisation improved and tissue damage was reduced following treatment. Regular exercise training has also been shown to augment the number of circulating EPCs in patients with cardiovascular risk factors and coronary artery disease, and is associated with improved vascular function and nitric oxide synthesis [48].

### CIRCULATING PROGENITOR CELLS AND LUNG DISEASES

Despite this understanding, little is known about circulating EPCs in lung diseases (table 1). To date, in asthma, for example, STIRLING *et al.* [49] have reported that systemic interleukin (IL)-5 increased circulating eosinophil progenitor

**TABLE 1** Comparison of the main roles of circulating endothelial progenitor cells (EPCs) in lung diseases

<b>Asthma</b>	Favourable role in asthma progression [49–52]: mobilisation, terminal differentiation and maintenance of mature eosinophil progenitors
<b>PH</b>	Promote PH? [53–55] Protective role against PH? [56] No effect because of EPC dysfunction? [57] EPC administration acts positively against PH in MCT-induced model [57, 58]
<b>COPD</b>	Favourable role in COPD progression: decreased tissue repair and/or angiogenesis? [52, 59, 60]

PH: pulmonary hypertension; COPD: chronic obstructive pulmonary disease; MCT: monocrotaline.

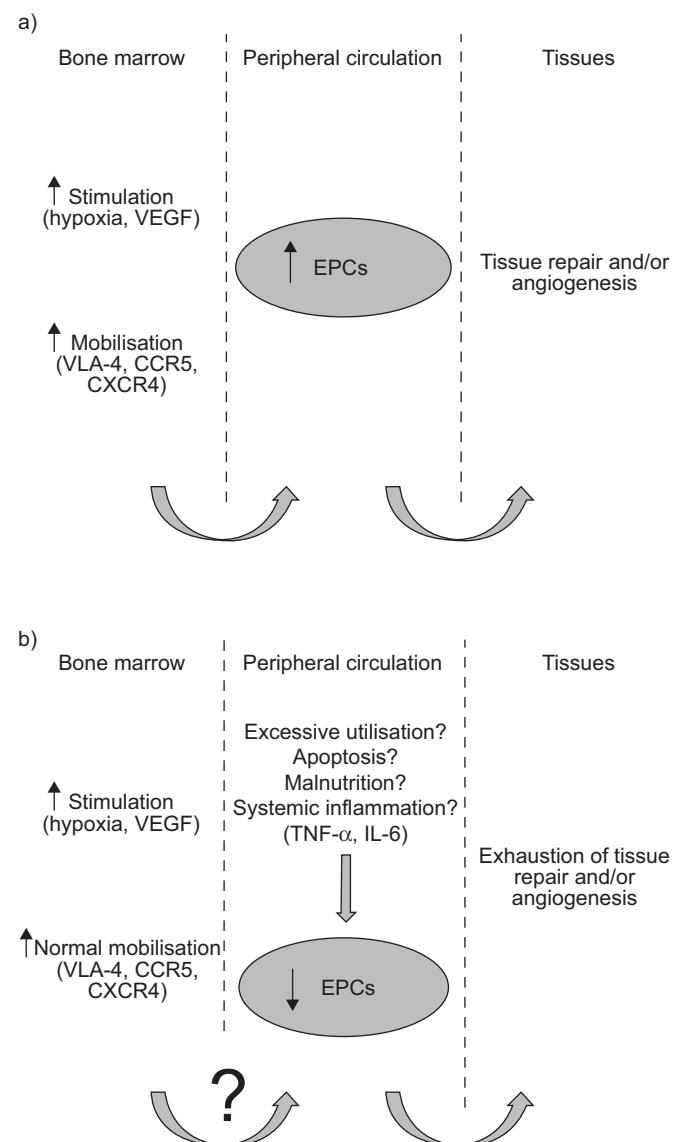
numbers, suggesting a key role for systemic IL-5 in eosinophil mobilisation. It is known that the inflammation of chronic asthma is associated with activation of peripheral blood T-lymphocytes and eosinophils and subsequent infiltration of these cells into the airway [61]; in particular, type-2 T-helper cells, source of IL-5, are increased in the asthmatic airway [62]. IL-5 levels have been measured in the circulation of asthmatic individuals [63], rise during asthma exacerbations [64] and play an important role in the mobilisation, terminal differentiation and maintenance of mature eosinophils [65]. It has been shown that mature eosinophils develop from pluripotent haematopoietic progenitor cells that express the cell surface glycoprotein CD34 [50]. Increased numbers of CD34+ cells have been demonstrated in both blood and bone marrow from atopic individuals as compared with normal subjects [51], and in the bronchial mucosa of atopic asthmatic subjects [66]. These findings underlie the central role of IL-5, confirming the data of O'BYRNE *et al.* [67] on the mobilisation of eosinophil precursors (CD34+/CD45+ progenitors) into the peripheral blood, and that systemic rather than airway IL-5 activity may be more potent in this role.

In pulmonary diseases in which endothelial dysfunction represents a key part of the pathophysiology, growing evidence is showing the importance of EPCs. In idiopathic and associated pulmonary arterial hypertension (PAH), clinical studies have suggested a link between EPC count and PAH severity, but with controversial results, as recently reviewed elsewhere [52]. In experimental studies, EPC numbers are increased in hypoxia-induced PAH because of compensatory mobilisation and recruitment mechanisms. As a therapeutic tool, infusion of EPCs seems promising in monocrotaline-induced PAH, but further investigations are required for a better understanding.

### Chronic obstructive pulmonary disease

EPCs may also be involved in COPD, but little is known about their role in chronic bronchitis and emphysema. COPD is also frequently associated with abnormalities in the pulmonary circulation, leading to pulmonary hypertension and right ventricular failure, with a greater mortality risk [68]. PAH in COPD occurs due to alterations in pulmonary vessel structure (intimal hyperplasia of muscular arteries and muscularisation of small arterioles) [69]. Intimal hyperplasia is produced by the proliferation of poorly differentiated smooth muscle cells and the deposition of collagen and elastic fibres [69]. The molecular mechanism of mobilisation, homing and differentiation of putative smooth-muscle-like progenitor cells remains to be clarified. Since endothelium plays a key role in regulating cell growth in the vessel wall, it has been hypothesised that endothelial dysfunction might be an initiating event that promotes vessel remodelling and PAH in COPD [68]. However, the pathobiology of pulmonary vascular remodelling in COPD is not fully understood and it remains unclear whether endothelial-like cells and smooth-muscle-like cells differentiate from a common vascular progenitor cell. Several animal and clinical studies have demonstrated that transplantation of autologous EPCs or unfractionated bone marrow cells facilitates vascular repair [8, 70]. Conversely, others have suggested that circulating EPCs could participate not only in maintenance of vascular homeostasis but also in the pathogenesis of various

diseases, by inducing smooth muscle cell proliferation and neointimal formation at sites of vascular injury [71–74]. PEINADO *et al.* [53] showed the presence of vascular progenitor cells (VPCs) adhered to the endothelial surface and within the intimal layer in pulmonary arteries of COPD patients. The presence of VPCs in the vicinity of areas of endothelial denudation and their relation with the response to hypoxic stimulus suggested that these cells might contribute to an ongoing process of endothelial repair. The authors also described an increased number of VPCs in the intima of pulmonary arteries, which was associated with the enlargement of the vessel wall. The latter finding suggests that VPCs could also be involved in the pathogenesis of intimal hyperplasia, presumably through VEGF-related signals.



**FIGURE 1.** Bone marrow response to endothelial damage in: a) normal subjects; and b) chronic obstructive pulmonary disease patients: potential mechanisms and consequences. VEGF: vascular endothelial growth factor; VLA: very late antigen; CCR: CC chemokine receptor; CXCR: CXC chemokine receptor; EPC: endothelial progenitor cell; TNF: tumour necrosis factor; IL: interleukin.

Furthermore, TUDER *et al.* [75] and KASAHARA *et al.* [76] have shown that emphysema is associated with decreased lung expression of VEGF and its receptor VEGFR-2 in animal models. Therefore, it is reasonable to assume that EPCs might be involved in COPD pathogenesis and that the bone marrow could be a new target of COPD. It has been shown that 13% of COPD patients have anaemia associated with increased systemic inflammatory marker levels [77, 78], but few data are available regarding other cell lineages, despite neutrophil activation and increased smoke-related turnover of granulocytes [79]. Increasing evidence from our group, confirmed by others, is showing that circulating progenitor cells are involved in COPD and correlate with disease severity. It has been demonstrated that circulating haemopoietic progenitor cell numbers are greatly decreased at rest, do not increase after endurance exercise and correlate with the level of hypoxaemia, severity of airway obstruction and peak oxygen uptake in COPD patients [59]. These data suggest a possible involvement of EPCs in the pathogenesis of decreased exercise capacity. In particular, COPD patients may show decreased capability for repair or angiogenesis of skeletal muscle due to a reduction in the number of circulating CD34+ cells, perhaps secondary to excessive peripheral utilisation of these cells or activation of apoptosis. CD34+ cells were also shown to be involved in tissue repair in skeletal muscle: 1) CD34+ cells are able to migrate to muscular connective tissue [80]; and 2) in a murine model, the percentage of these progenitor cells increased in muscle fibres when animals were kept active (running wheel in the cage) [81], suggesting that CD34+ cells were capable of substituting skeletal muscle. Data have also been published on athletes, showing that circulating CD34+ cell counts were higher in runners than in sedentary subjects, but decreased on the day following a marathon race, in agreement with their possible utilisation in skeletal muscle for repair purposes [82]. Furthermore, the hypothesis that the type of exercise and fitness level could modulate cytokine/growth factor release has also been tested; indeed, it was found that all-out exercise in well-trained rowers acutely mobilised haematopoietic progenitor cells, T- and natural killer lymphocytes and immature reticulocytes, suggesting that progenitor cell mobilisation may occur in response to tissue hypoxia, or signals originating in skeletal muscle at a very high workload [83]. The lung could represent another hypothetical target for circulating EPCs in COPD patients. Examination of lung tissue from COPD patients revealed the presence of apoptotic cells in greater number than in control lungs or those from smokers without COPD [76]. Apoptotic cells included alveolar and bronchial epithelial cells, as well as ECs in the parenchyma. Importantly, the apoptosis persisted in patients with COPD after smoking had ceased [60]. It could be hypothesised that the excessive lung utilisation of circulating EPCs, in response to cell apoptosis, led to a decreased number of circulating EPCs (fig. 1). Further studies are still required in order to elucidate the targets of the circulating EPCs.

As for the mobilisation and homing of EPCs, it is possible that inflammatory processes, occurring in COPD (neutrophil activation and oxidative stress), or tissue hypoxia may be involved in the decrease in circulating CD34+ cell numbers, but little is known regarding possible associations between circulating CD34+ cells and pro-inflammatory mediators in

COPD. In severe COPD patients who displayed a low body mass index (BMI), the bone marrow appears insufficiently stimulated despite higher plasma growth factor concentrations [84]. Therefore, it is reasonable to speculate that the bone marrow could be inhibited by myelosuppressive cytokines, such as tumour necrosis factor- $\alpha$ , leading to reduced regenerative capacity. It has recently been shown that disease severity correlated with pro-angiogenic and inflammatory marker levels and was inversely associated with circulating EPC numbers [84]. Furthermore, among patients with similar pulmonary impairment, those who displayed a low BMI had a more markedly reduced number of circulating EPCs. The findings also indicate that, in severe low-BMI COPD patients, bone marrow function seems to be further impaired and may lead to reduced reparative capacity. The systemic involvement of COPD, characterised by systemic inflammation, malnutrition and skeletal muscle dysfunction, may indicate exhaustion of repair and/or angiogenic resources (fig. 1) [85].

## CONCLUSION

As revealed in the present review, several potential uses of bone marrow-derived circulating EPCs are indicated. The regulation of EPC homing, differentiation and proliferation remains unclear and requires greater understanding, in particular in chronic lung diseases, in order to achieve optimal therapeutic benefits. As for cardiovascular diseases, therapeutic utilisation of EPCs might also be extended to COPD in the future.

More studies are needed to understand the pathophysiology of chronic lung diseases, such as COPD, particularly regarding the systemic involvement of the disease.

## STATEMENT OF INTEREST

None declared.

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