

Endothelial progenitor cells in 2006 — where are we now?

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Background

The vascular endothelium, long thought of as a static barrier between tissue and blood, actually plays a major role in cardiovascular biology. The endothelium regulates vascular form and function, transport of solutes to tissue and the anti-thrombotic interface between tissues and circulating blood. It is known that defects in endothelial function or structure are hallmarks of vascular diseases, including systemic and pulmonary hypertension, atherosclerosis and restenosis in response to vascular injury. The endothelium undergoes a constant process of injury and repair in response to mechanical and chemical injuries.¹ Emerging evidence suggests that bone marrow-derived endothelial progenitor cells (EPCs) contribute to the repair of vascular injury and play a role in tissue repair.

Circulating cells that may contribute to re-endothelialisation include mature endothelial cells (sloughed) as well as cells capable of differentiating into endothelium. As early as 1963, Stump et al provided evidence for the existence of circulating sources of endothelium.² In an animal model, a small patch of Dacron was suspended within the lumen of an aortic interposition graft that was also made of prosthetic material. The Dacron patch, floating midstream, was thus isolated from contact with native vascular tissue. After seven days, islands of endothelial cells were identified on the patch surface, leading the authors to postulate circulating blood as the endothelial source.

Interest in the concept of a circulating source of endothelium was renewed in the late 1990s. Asahara et al reported isolation of EPCs from CD34+ selected peripheral blood mononuclear cells.³ This population of cells contained only 15% CD34+ cells and, when cultured on fibronectin, developed phenotypic characteristics of endothelial cells. These cells incorporated into sites of angiogenesis in a rabbit model. Later, the same group generated similar EPCs from non-selected human peripheral blood monocytes (PBMCs). After seven days in culture, over 90% of these cells expressed CD14, a monocyte marker, in addition to expressing flk-1, VE-cadherin and CD31, traditional endothelial cell markers. The term 'endothelial

progenitor cells' was introduced at this stage, but indeed this was probably a misnomer, as these cells are not true stem cell progenitors, but exhibit both an endothelial and monocytic phenotype. As regards their biological properties, these cells are senescent in nature.

Lin et al, using peripheral blood cells from gender-mismatched bone marrow transplants and distinct culture conditions, distinguished the presence of sloughed, senescent, mature, recipient-derived endothelial cells and donor-derived cells able to generate a robust population of blood outgrowth endothelial cells (BOECs).⁴ These studies showed that indeed bone marrow-derived cells enter the peripheral circulation and have an endothelial phenotype. BOECs take up acetylated low density lipoprotein (LDL), and express vWF, flk-1 and VE-cadherin, all traditional endothelial cell markers as mentioned above.

Of note, these BOECs did not express CD14, distinguishing them from EPCs identified in the earlier studies. Peichev and others have further identified peripheral CD34+ cells that express CD 133 and VEGFR2, which may be functional BOEC precursors.⁵ Therefore this demonstrates that these cells, without the CD14 monocyte marker, were able to grow out into true bona fide endothelium in the presence of culture conditions, suggesting the CD14-negative fraction of cells possibly contain the true EPCs.

However, the role of CD34+ cells as the sole source of endothelial cells has been challenged. The observation that this enriched EPC population commonly expressed CD14 led investigators to study the role of CD34-/CD14+ cells in the development of endothelial cells. Schmeisser et al have shown that CD34-/CD14+ cells can develop an endothelial phenotype in culture.⁶ Harraz et al also demonstrated that a subset of CD34-/CD14+ cells can assume an endothelial-like phenotype in culture and incorporate into the endothelium of angiogenic sites.⁷ Interestingly, the addition of CD34+ cells to this population was required for the incorporation of CD34-/CD14+ cells suggesting intercellular co-operation in angiogenesis.

Based on the preponderance of evidence, we conclude that the population of circulating cells with an endothelial phenotype, or

potential of assuming an endothelial cell phenotype, is heterogeneous and includes mature sloughed endothelial cells, monocytes (CD14+) capable of phenotypic and functional differentiation and, importantly, a subset of CD34+ 'true' endothelial precursors that likely express CD133 and VEGFR2 as well. Therefore, it is fair to say that bone marrow-derived cells in the circulating blood have an endothelial phenotype and also peripheral blood can be cultured to generate endothelial cells.

Endothelial progenitor cells as biomarkers for cardiovascular disease

In recent years, data have emerged that circulating vascular progenitor cells provide both diagnostic and prognostic information with respect to cardiovascular disease. As a biomarker, EPCs are analysed by their phenotypic markers, as discerned by fluorescence-activated cell sorting (FACS) analysis and also by their functional capability to produce colonies in culture conditions.

The importance of EPCs in repairing vascular damage in the clinical setting remains unknown. Indeed EPCs may play multiple roles in the vascular repair process. Of note, observations in type II diabetes, a condition that is associated with endothelial dysfunction, have shown that recruitment of endothelial progenitors to the site of tissue repair is diminished⁸ and that statin therapy⁹ promotes the mobilisation and the function of progenitor cells. Evidence now also exists that EPCs may act as a serum marker for vascular disease.¹⁰

A landmark paper by Hill et al in 2003 looked at EPCs with respect to their functional ability to produce colony-forming units. Peripheral blood from patients with increased brachial reactivity as a marker of health showed increased production of colony-forming units under growth culture conditions *in vitro*. Of note, those patients with an increased Framingham Risk Score produced less colony-forming units *in vitro*.¹¹

Werner et al have recently published a FACS analysis study on patients attending for cardiac catheterisation and phenotypic markers such as CD34+ and VEGFR2 were measured. Results pointed to patients with the higher number of cells with these markers as having lower cardiovascular mortality.¹² This is backed up by another study by Schmidt et al where, again, patients with a higher number of cells with these markers have an increased event-free survival.¹³

Why is an increased number of EPCs with these markers associated with a better cardiac outcome? The reason is still not clear. Perhaps these patients have less unstable plaque and, therefore, the circulating vascular progenitors are higher in number (as they are not recruited to the arterial wall).

Currently no theory for this finding has been proven. Ongoing work is continuing in terms of EPCs in acute myocardial infarction and in patients who have coronary endothelial dysfunction upon intracoronary acetylcholine administration without an actual coronary atherosclerotic

burden. This area of EPC biology is still incompletely understood and many more questions need to be answered.

Therapeutic applications of EPCs

Induction of angiogenesis

To date, the primary therapeutic application for bone marrow and circulation-derived progenitors has been based on their angiogenic potential. Pre-clinical animal studies demonstrating angiogenic effects of EPCs have led to the establishment of phase one clinical trials using intracoronary delivery of auto-logous peripheral blood-derived cells. Most of these studies are aimed at delivering these multi-potent cells to injured myocardium.

Following myocardial infarction (MI), eight studies have shown therapeutic effects on left ventricular form and function. The recently published final one year follow up results for a phase one clinical trial by Assmus et al¹⁴ showed sustained improvement in left ventricular function, reduction in systolic volumes and smaller infarct sizes in the two groups of patients treated with cell therapy.

Wollert et al published the first randomised, controlled trial to assess the effect of intracoronary transfer of autologous bone marrow cells on left ventricular functional recovery in patients after acute MI and successful percutaneous coronary intervention (PCI).¹⁵ Sixty patients were randomly assigned to either a control group (n=30) that received optimum post-infarction medical treatment or a bone marrow cell group (n=30) that received optimal medical treatment and intra-coronary transfer of autologous bone marrow cells 4.8 days after PCI. Even though patient numbers are small in this study, it shows that infusion of autologous bone marrow CD34+ cells into the infarct-related coronary artery during the early post-infarction period improves recovery of global left ventricular ejection fraction (LVEF) after six months as determined by cardiac magnetic resonance imaging (MRI).

However, not all of these post-MI trials have shown an improvement in left ventricular function. In early January 2006, *The Lancet* published a randomised, double-blind, placebo-controlled trial of stem cell therapy for restoration of ventricular function after MI. The study was well designed with a blinded, placebo-controlled outline, but unfortunately there was no improvement in cardiac function. Four months after infarction, ejection fraction increased by 2.2% in control patients and 3.4% in patients who received stem cells. The lack of improvement was thought to be perhaps due to the fact that the ventricular function was too well preserved to expect functional improvement from stem cell infusion.¹⁶

In the chronic setting, autologous cells have been injected directly into dysfunctional myocardial segments to enhance contractile function. Stamm et al injected autologous AC133+ bone marrow cells into the infarct border during coronary artery bypass grafting in six patients who had experienced earlier MI. Results showed improved perfusion of the infarcted area and

significant enhancement of global left ventricular function three to nine months after surgery and no incidence of adverse events in five of the six patients.¹⁷

Lower limb angiogenesis trials have also been performed.¹⁸ Autologous bone marrow-derived cells were injected into the gastrocnemius muscle of patients with unilateral or bilateral leg ischaemia caused by severe peripheral artery disease not amenable to any further revascularisation procedures. During the 24 week duration of the study, ankle brachial indexes were significantly improved in the legs of patients treated with bone marrow-derived cells, but not in patients treated with saline. Rest pain and pain-free walking also improved significantly during the study.

Large vessel repair

With the rediscovery of the circulation as a source of endothelial cells, multiple studies have been performed highlighting the potential use of bone marrow-derived cells to enhance vascular healing at sites of vascular injury. The first of these studies was performed in a splenectomised mouse model. Following systemic delivery, cultured spleen-derived mononuclear cells homed to injured arterial segments. Cell administration was also associated with accelerated re-endothelialisation and reduced neointimal formation following injury.¹⁹ Fujiyama and et al have shown that targeting of bone marrow-derived mononuclear cells (CD34-/CD14+) can be stimulated by genetically elevated levels of monocyte chemo-attractant protein-1 (MCP-1) to adhere and promote healing of injured carotid arteries in nude rats.²⁰

Multiple groups have demonstrated the potential use of circulation-derived endothelial cells to improve vascular healing using more direct models of vascular injury and autologous cell delivery. Gulati et al generated cells with a partial endothelial phenotype from peripheral blood following seven days of culture called culture-modified mononuclear cells (CMMCs). These are indeed similar to EPCs. Balloon injury to an isolated segment of carotid artery was performed, followed by intraluminal administration of autologous CMMCs. Four weeks later, CMMCs delivery was associated with a 55% reduction in neointimal thickening and significantly enhanced arterial re-endothelialisation.²¹ Of note, labelled cells made up a vast minority of resident cells four weeks following injury, suggesting a paracrine role for these cells. As the translation of pre-clinical to clinical studies takes place, the concept of 'dual delivery' of cells must be analysed closely. In vitro manipulation of cells under specialised growth conditions leads to 'early' cells at days four to seven (as mentioned above) with the CMMCs and 'late' cells at days 21-28 with true outgrowth endothelial cells. Creating an ideal clinical product for treatment, one would envisage that earlier cells would be translationally much more pragmatic, but they must also have functional properties, which may take time to develop *ex vivo*. The length of time in going from bench to bedside for these cells is still uncertain.

Delivery of genetically modified autologous endothelial cells would provide for enhancement of defined paracrine effects or establishment of new therapeutic approaches. EPCs could be genetically modified to overexpress antithrombotic, vaso-dilatory or antiproliferative genes to improve the function of the implanted cells in injured vessels or prostheses and to prevent thrombosis and restenosis. Kong et al have shown that the therapeutic effects of EPCs could be enhanced by over expressing endothelial nitric oxide synthase (eNOS).²² Denuded carotid arteries treated with the eNOS-expressing EPCs had enhanced inhibition of neointima and reduced incidence of thrombosis, compared with vessels treated with control vector. This is only one example where cells have been transduced to overexpress proteins normally expressed, or to express new proteins in endothelial cells to enhance their native effects.

Drug-eluting stents (DES) have revolutionised coronary artery revascularisation and have practically eliminated restenosis in many settings. Delayed endothelialisation is a feature of DES and prolonged dual antiplatelet therapies are required to prevent stent thrombosis. Higher risk for restenosis even with DES is seen in certain patient categories such as diabetics, small vessels and long lesions. The results of the HEALING I, II and III trials, which began recruiting patients in late 2003, hold exciting potential for the future. These studies used the Genous stent, which is coated in CD34 antibody which then binds to the CD34 antigen of circulating EPCs promoting re-endothelialisation in minutes to hours. Unlike the 'anti-tumour' restenosis prevention of DES, this mechanism seeks to restore the normal biology of the vessel wall rather than perpetuate its disruption.

Conclusion

As we make our way through 2006, the importance of the circulation-derived vascular cells, especially the EPC population, has already been firmly established. I foresee a larger number of small clinical trials continuing to be performed, but it must be stressed that these trials might only detect safety concerns and would not be expected to test efficacy. Clinical studies must be carried out in collaboration with basic science investigators who are trying to unravel and optimise the biology underlying this therapeutic approach.

The role of EPCs as cardiovascular biomarkers is still at an experimental stage, but the growth and development in this area will no doubt be exponential. There are many questions still unanswered regarding the definition of the 'true EPC' and the proportion of EPCs circulating in the acute and chronic cases of coronary ischaemia and indeed in left ventricular dysfunction. Whether these cells are involved in the inflammatory response still needs to be elucidated.

Finally, the main part of the puzzle will be attempting to define the exact biological phenotype of the true EPC and discerning the exact mechanism of action of these cells on the endothelium. Is the EPC autocrine or paracrine in its

mechanistic role? We are still uncertain.

As Yeats recited: "O body swayed to music, O brightening glance, how can we know the dancer from the dance."

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