Viewpoint

It's all in the blood: circulating endothelial progenitor cells link synovial vascularity with cardiovascular mortality in rheumatoid arthritis?

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The belief that there exists an undifferentiated cell which can be programmed into another type of cell forms the basis of stem cell biology as we know it today. This has opened up possibilities of new treatment approaches for a whole host of diseases. In the field of vascular biology there is also thought to be a renewable source of cells present in the adult that have the potential to develop along either the haematopoietic or the endothelial lineages. Fascinatingly, recent studies have uncovered evidence suggesting that in rheumatoid arthritis (RA) such endothelial progenitor cells (EPC) are not only involved in synovial vascularisation, but may also underlie the increased cardiovascular morbidity and mortality known to be a feature of RA, thus linking two features of the disease that are well characterised but are as yet poorly understood.

The notion that angiogenesis – the formation of new blood vessels from pre-existing vasculature – is important in the perpetuation of RA synovitis is no longer novel. There is ample evidence supporting this idea, ranging from expression of proangiogenic factors such as vascular endothelial growth factor (VEGF) to studies in which inhibition of angiogenesis reduced the disease severity in animal models of arthritis [1]. However, while the rheumatologic and scientific community is in all probability now quite familiar with angiogenesis and its role in RA pathogenesis, the contribution made by EPC to blood vessel formation is less well understood. The distinct processes that result in the formation of the vasculature are vasculogenesis, which is the coalescence of endothelial cells, angiogenesis, and arteriogenesis, when the lumen of vessels increases to form collateral arteries. In the embryo, development of the vascular system occurs via a combination of vasculogenesis and angiogenesis. Formation of the primordial vascular network results from the commitment by precursor cells (haemangioblasts) to form endothelial cells, rather than haematopoietic stem cells. Following *in situ* differentiation of these EPC to form clusters of endothelial cells (or blood islands), these cells then multiply and interconnect to give rise to the yolk sac capillary network. Mature vessels then form by budding and re-modelling of pre-existing vessels. VEGF and its receptors are intimately involved in the regulation of both embryonic vasculogenesis and angiogenesis.

Vasculogenesis has now been shown to be recapitulated in adults postnatally. EPC were first isolated from human peripheral blood by selection for cells expressing the haematopoietic maker CD34 [2]. These cells were found to differentiate into endothelial cells and to express both haematopoietic markers such as CD133 and endothelial cell markers, including CD31 and VEGF receptor type 2. Crucially, these cells also incorporated into sites of angiogenesis *in vivo* [2]. These observations were the first to suggest that blood vessels in adults may not only arise by angiogenesis, but may also result from recruitment to blood vessels of a progenitor population (vasculogenesis). This was further expanded upon in studies demonstrating that VEGF, which was well described as playing a central role in diseases associated with alterations in vessel density, can mobilise EPC from the bone marrow [3,4]. VEGF can thus be considered a bifunctional stimulus for new blood vessels, promoting endothelial proliferation (angiogenesis) and EPC mobilisation (vasculogenesis). EPC have also been recently described with reference to RA. CD34/VEGF receptor type 2-positive and CD133/VEGF receptor type 2-positive cells were found in apposition to RA synovial blood vessels [5]. Bone marrow-derived CD34-positive cells were subsequently expanded into CD31-expressing cells and von Willebrand factor-expressing cells. Interestingly, these cells were generated at a higher rate from bone marrow samples taken from RA patients, compared with normal subjects. Furthermore, the capacity of bone marrow-derived cells from RA patients to progress into endothelial cells correlated with the synovial microvessel density [6]. It thus appears that EPC

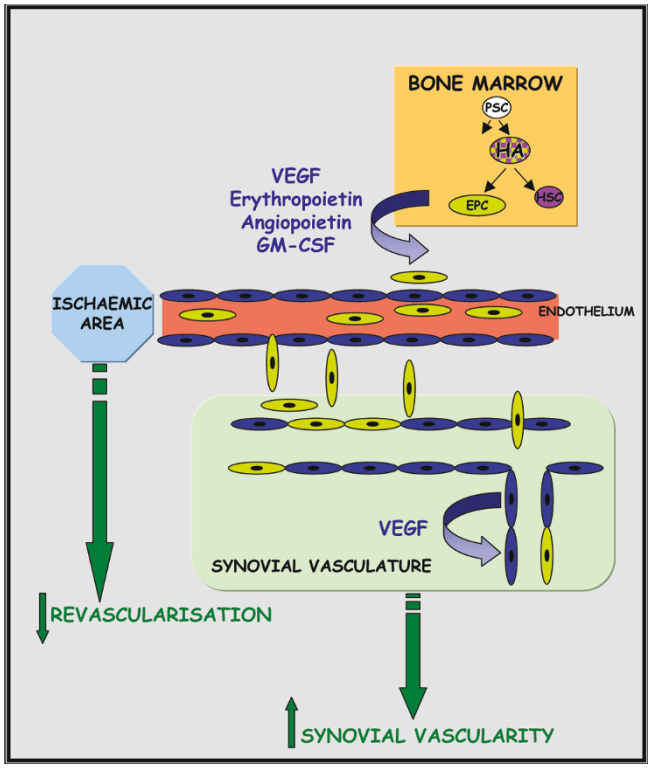
EPC = endothelial progenitor cells; RA = rheumatoid arthritis; TNF-α = tumour necrosis factor alpha; VEGF = vascular endothelial growth factor.

are present in RA synovium, and indeed that their generation from bone marrow is enhanced.

Two very recent studies have reported that EPC numbers are actually decreased in the peripheral blood of RA patients compared with healthy individuals. An elegant study from the group of Josef Smolen showed that EPC levels in patients with active RA were significantly lower than in individuals with inactive disease or healthy controls, and that they correlated inversely with disease activity. Moreover, treatment of patients with active RA using tumour necrosis factor alpha (TNF-α) inhibitors restored circulating EPC levels to those seen in healthy control subjects. What was also interesting was the fact that patients on conventional disease-modifying antirheumatic drug therapy or low-dose steroid therapy did not show normalisation of circulating EPC numbers [7]. The observation of reduced circulating EPC in patients with RA was confirmed in another study by Herbrig and colleagues, who also demonstrated reduced migration of EPC from RA patients in response to VEGF. Interestingly, EPC from RA patients exhibited only modest adhesion to endothelial cells stimulated with TNF-α, compared with EPC from healthy subjects, despite comparable levels of adhesion to unstimulated endothelial cells or matrix proteins such as laminin [8].

What is the relevance of these findings in terms of our understanding of RA? Increased angiogenesis is thought to be crucial in supplying nutrients and oxygen to the synovial tissue in RA, which by virtue of its proliferative nature becomes rapidly hypoxic [1]. The presence of EPC in RA synovium could result from their enhanced recruitment from peripheral blood. This might then lead to increased RA synovial blood vessel formation, perpetuating disease. Furthermore, increased EPC trafficking to the synovium would account for the observations of reduced peripheral blood EPC in RA. However, the corollary of augmented synovial tissue EPC paralleled by reduced circulating EPC might also be an impaired ability to revascularise areas of ischaemia. There has certainly been considerable excitement in the cardiovascular field in recent years about the potential for mobilising bone marrow-derived EPC to increase neovascularisation for the treatment of ischaemic disease [9,10]. The awareness that angiogenesis is pertinent in the context of cardiovascular disease has arisen from the fact that occlusion or narrowing of arteries is likely to result in hypoxia, in response to which the ischaemic myocardium develops collateral vessels. This compensatory angiogenesis seems to be insufficient, however, resulting in the need to perform surgical revascularisation procedures. The concept of therapeutic angiogenesis exploits and supplements the physiological response to hypoxia/ischaemia. There have been reports of enhanced ischaemic limb perfusion following injection of bone marrow-derived mononuclear cells. Indeed, the first description of EPC showed that peripheral blood-derived CD34-positive cells integrated into newly formed vessels in an *in vivo* model of limb ischaemia [2].

Figure 1



Pluripotent stem cells (PSC) in the bone marrow give rise to haemangioblasts (HA), with the potential to differentiate into either haematopoietic stem cells (HSC) or endothelial cell precursors (EPC; green). Mobilisation of EPC from the bone marrow is upregulated by many factors, including vascular endothelial growth factor (VEGF), erythropoietin, angiopoietin-1, and colony-stimulating factors such as granulocyte–macrophage colony-stimulating factor (GM-CSF). In rheumatoid arthritis (RA), these cells appear to traffic to RA synovium at an enhanced rate, incorporating into blood vessels and giving rise to increased vascularity – thereby reducing the potential for revascularisation of ischaemic areas. Angiogenesis in the synovium is also VEGF dependent. As a consequence, circulating EPC numbers are reduced in RA and may lead to increased cardiovascular mortality.

This is an important issue in the context of RA, since an increased risk of cardiovascular disease, mainly due to accelerated atherosclerosis, is well known to be associated with RA. In addition to a role for the classical risk factors such as raised levels of low-density lipoprotein cholesterol, there is also strong evidence for endothelial dysfunction in RA. This was certainly seen in the study of Herbrig and colleagues, who studied blood flow in the forearm following infusion of acetylcholine. Vasodilatation was significantly reduced in the arms of RA patients [8]. The inference is therefore that reduced circulating EPC in patients with active RA would result in a poorer response to ischaemia, and thereby cardiovascular events such as stroke or myocardial infarction. This is supported by the findings of Smolen and colleagues' study, which quantified the angiogenic potential of EPC by measuring the binding of *Ulex europaeus* lectin and the

uptake of acetylated low-density lipoprotein. Cells positive for these attributes of angiogenic EPC were reduced when expanded from patients with active RA, compared with inactive or healthy controls [7].

What conclusions do the findings of EPC in RA synovium and reduced circulating EPC leave us with? Firstly, it seems probable that increased synovial vessel density in RA is not just due to angiogenesis, but also to postnatal vasculogenesis, owing to mobilisation of EPC from the bone marrow. Secondly, it would seem also that the increase in EPC homing is paralleled by reduced circulating EPC, which could significantly contribute to the cardiovascular co-morbidity of RA (Fig. 1). VEGF has long been thought of as a 'bad guy' in RA and as a possible therapeutic target, but now it seems that VEGF inhibition might also affect EPC. It is clear, however, that this may work in one opposing way or even both opposing ways – reducing EPC recruitment to the synovium and/or downregulating EPC mobilisation from the bone marrow. This would obviously have different outcomes, the former increasing circulating EPC and the latter decreasing circulating EPC. Moreover, the demonstration that TNF-α inhibition led to restoration of circulating EPC levels in RA patients suggests not only that these biological therapies modulate disease itself, but that they may also be beneficial in terms of normalising the cardiovascular risk associated with this disease – yet another advantage of TNF-α blockade!

Taken together, it would seem that EPC and their involvement in postnatal vasculogenesis might connect different features of RA; namely, increased vascularity leading to synovial expansion and cardiovascular co-morbidity.

Competing interests

The author(s) declare that they have no competing interests.

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