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Endothelial Progenitor Cells: New Targets to Control Autoimmune Disorders

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1. Introduction

The formation of blood vessels is essential for preparing a closed circulatory system in the body, and for supply of oxygen and nutrients to all tissues and organs. One of the key mechanisms behind many autoimmune diseases is abnormal blood vessel structure and function. This dysfunction is reflected in some of the serious manifestations of rheumatoid arthritis (RA), type 1 diabetes mellitus (T1DM) and systemic sclerosis (SSc) that are currently difficult to treat, such as loss of fingers due to reduced blood flow, kidney failure due to renal hypertensive crisis and heart failure due to pulmonary arterial hypertension. The cells that line blood vessels (endothelial cells) not only confine blood to the vessels but actively participate in the recruitment of circulating cell subsets to sites of inflammation and vascular permeability for the exchange of solutes and gases. Collectively, endothelial cells play many roles in the development and maintenance of blood vessels. Blood vessel development occurs primarily via one of two mechanisms, angiogenesis (the generation of blood vessels from pre-existing vasculature) and vasculogenesis (the recruitment of endothelial progenitor cells from the bone marrow to sites of vascularisation). In recent decades, extensive studies have revealed that a variety of factors and their receptors regulate angiogenesis in vertebrates, including vascular endothelial growth factor (VEGF)-VEGFRs, angiopoietin-Tie, Ephrins-EphRs and Delta-Notch (reviewed by Karamysheva (Karamysheva, 2008)). Indeed, targeting these molecules has resulted in significant advances in the treatment of cancer and cardiovascular disease. However, the burden of diseases that involve vascular dysfunction is immense and continues to rise with drug resistance, intolerance and ineffectiveness being significant contributors. Less is known about the mechanisms underpinning vasculogenesis and despite an explosion of research in this area over the past decade we are yet to fully exploit these cells for therapeutic benefit (Sen et al., 2011, Sieveking and Ng, 2009). This chapter discusses whether the endothelial progenitor cells (EPCs) from patients with autoimmune diseases, such as RA, T1DM and SSc, behave differently from normal EPCs and whether there are factors in the serum of these patients that may be responsible for this abnormal behaviour. The altered behaviour of EPCs in patients with autoimmune disease is poorly understood, based on limited studies to date. This chapter addresses whether EPCs would be a prime target for therapeutic intervention in the serious complications of autoimmune disease.

2. Vascular dysfunction in autoimmune disease

2.1 Rheumatoid arthritis

RA is a chronic and debilitating autoimmune disease that affects the joints. The disease is characterised by inflammation of the synovial tissue, which lines the joints and tendons. In healthy tissue, the synovium is made up of synovial cells, a network of capillaries and lymphatic vessels, and a well-organized matrix containing proteoglycan aggregates. Between the cartilage and synovium is the synovial fluid, which nourishes and lubricates the joint. In RA, cells of lympho-haematopoietic origin, e.g. T-helper cells, B cells and macrophages, infiltrate the synovium. The synovium also becomes thickened, from a layer of 1–2 cells to approximately 6–8 cells, and becomes locally invasive at the interface with the cartilage and the bone or tendon. The volume of the synovial fluid eventually increases in volume as a result of oedema, which causes swelling of the joints and pain.

Several lines of evidence indicate that RA is associated with aberrant and severe vasculogenesis (i.e. the de novo formation of blood vessels) within the inflamed joints (Paleolog, 2009, Grisar et al., 2007, Grisar et al., 2005, Herbrig et al., 2006, Hirohata et al., 2004, Jodon de Villeroche et al., 2010, Ruger et al., 2004, Silverman et al., 2007). One of the first observations of vasculogenesis in RA was the discovery that the synovial fluids from patients with RA contained a low molecular weight vasculogenesis factor apparently identical to that derived from tumours (Brown et al., 1980). Subsequent studies revealed that synovial fluid from patients with RA stimulated proliferation of human endothelial cells (Kumar et al., 1985) and the formation of tubular networks (Semble et al., 1985). A study of synovial tissue histology from patients with RA revealed that there is a significant correlation between the number of synovial blood vessels and vessel proliferation, mononuclear cell infiltration, fibrosis and clinical measurements of joint tenderness (Rooney et al., 1988). Capillaries are distributed more deeply in the synovium from patients with RA (Stevens et al., 1991). The different stages of rheumatoid arthritis are shown in Figure 1 (upper panel). Although perivascular mononuclear cell infiltration and increased thickness of the synovial lining layer are observed in tissue from both inflamed and non-inflamed joints of RA patients, vascular proliferation is seen only in tissues from inflamed joints (FitzGerald et al., 1991). In addition, endothelial cells lining blood vessels within RA synovium have been shown to express cell cycle-associated antigens such as proliferating cell nuclear antigen and Ki67, and integrin alpha 5 beta 3, which is associated with vascular proliferation (Ceponis et al., 1998). Hypoxia, which can activate vasculogenesis factors and cause further invasion of the synovium, is another common event that occurs within the synovial joints in RA (FitzGerald et al., 1991, Muz et al., 2009). Taken together, these studies indicate that vascular dysfunction in synovial tissue is a likely therapeutic target in RA.

2.2 Type 1 diabetes mellitus

T1DM is a life-long autoimmune disease characterised by hyperglycaemia. Hyperglycaemia in T1DM occurs when the number of insulin-producing ß-cells in the pancreatic Islets of Langerhans drops below the number required to control glycaemia. Hyperglycaemia leads to macrovascular complications, such as coronary artery disease, peripheral arterial disease, and stroke, and microvascular complications, such as diabetic nephropathy, neuropathy, and retinopathy. Onset is early in life and patients exhibit increased risks of renal failure,

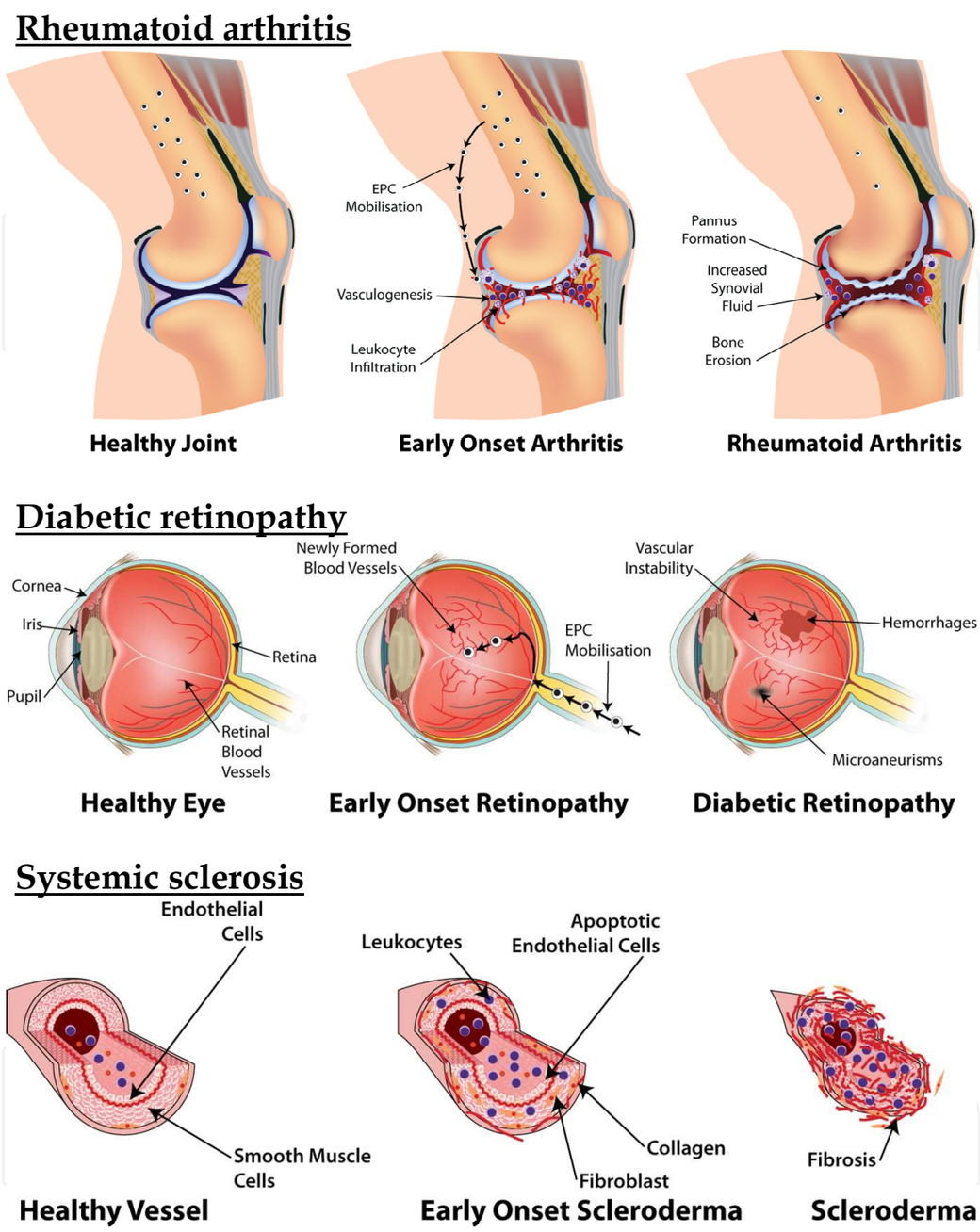
blindness, amputation, stroke and heart attack (Shapiro et al., 2006). Best available practice with insulin therapy is not a cure as it does not protect the remaining islets from inflammatory attack or the patient from long-term complications.

Insulin-producing ß-cells, which comprise 60-80% of islet mass, are crucial for the maintenance of normal blood sugar. Pancreatic islets are highly metabolically active and densely vascularised with specialized endothelium – they receive 10% of pancreatic blood flow despite comprising only 1% of tissue mass. Pancreatic islets come under a myriad of cellular assaults during isolation including ischemia, enzymatic damage and physical stress. Dysfunction of the endothelium plays a critical role in the development of vascular complications in T1DM (Stehouwer et al., 1997, Flyvbjerg, 2000). Clinical trials have shown that hyperglycaemia leads to changes in the proliferation of endothelial cells, barrier function and the adhesion of other circulating cells to endothelial cells (Schalkwijk and Stehouwer, 2005). This vascular dysfunction may be mediated by several distinct mechanisms and different stages of diabetic retinopathy are shown in Figure 1 (middle panel). Hyperglycaemia results in an increase in intracellular glucose, which leads to an increase in the conversion of glucose to sorbitol via the polyol pathway. This increase in sorbitol can cause osmotic stress, tissue hypoxia and oxidative stress (Williamson et al., 1993, Schalkwijk and Stehouwer, 2005). Hyperglycaemia also results in activation of protein kinase C, which can cause dysregulation of vascular permeability and blood flow, basement membrane thickening and impaired fibrinolysis (Williamson et al., 1993, Chen et al., 2000). In addition, hyperglycaemia causes increased glucosamine-6-phosphate and consequently increased transcription of cytokines such as transforming growth factor beta, which can regulate the proliferation and apoptosis of endothelial cells (Nerlich et al., 1998, Ziyadeh, 2004). Greater insight into the mechanisms underlying endothelial dysfunction may lead to important treatment strategies which can reduce the morbidity and mortality rate caused by endothelial dysfunction in patients with T1DM.

2.3 Systemic sclerosis

SSc is a heterogeneous disease in which vascular dysfunction, extensive fibrosis and autoimmunity are the hallmark characteristics. The aetiology of SSc is unknown as there are many unresolved questions as to both cause and initiating factors (Geyer and Muller-Ladner, 2011). Multiple genetic and environmental factors, combined with other specific factors (e.g. alterations to the immune system, vasculature and extracellular matrix) are the most likely causes of this insidious disorder. The pathophysiology of SSc is diverse and includes abnormal immunologic processes such as cytokine and chemokine dysregulation, abnormal T cell signalling, B cell dysfunction, endothelial injury, aberrant wound healing due to dysregulation of matrix homeostasis, abnormalities in the fibrinolytic system, polymorphisms in critical molecules of the immune system and matrix homeostasis, and microchimerism due to foetal/maternal placental exchange of HLA compatible cells (Gabrielli et al., 2009).

Vascular dysfunction is an early event in SSc (Kahaleh, 2008) and the different stages of SSc are shown in Figure 1 (lower panel). The preferred site of early lesions in SSc is the perivascular space. Progressive wall thickening and perivascular infiltrates are features of the vascular lesions in this compartment, indicating the involvement of vascular smooth-muscle cells and pericytes. Endothelial cells are the only type of mesodermal cell that



In rheumatoid arthritis (upper panel), circulating endothelial progenitor cells (EPCs) and vasculogenesis are causally linked to the influx of pro-inflammatory **leukocytes** and increased capillary beds contribute to thickening of the synovial lining and joint pain. In diabetic patients with proliferative retinopathy (middle panel), infiltrating EPCs contribute to the dense vascularisation in the eye and reduced vascular stability associated with blindness. Vascular injury is one of the early events in the pathogenesis of systemic sclerosis (lower panel) and is characterized by endothelial-cell damage and apoptosis, the proliferation of fibroblasts, production of collagen and **infiltration** of circulating leukocytes. Despite the increased number of circulating EPCs in these patients, the endothelial layer of the vasculature remains denuded and is ultimately obliterated.

Fig. 1. Vascular dysfunction in autoimmune disease.

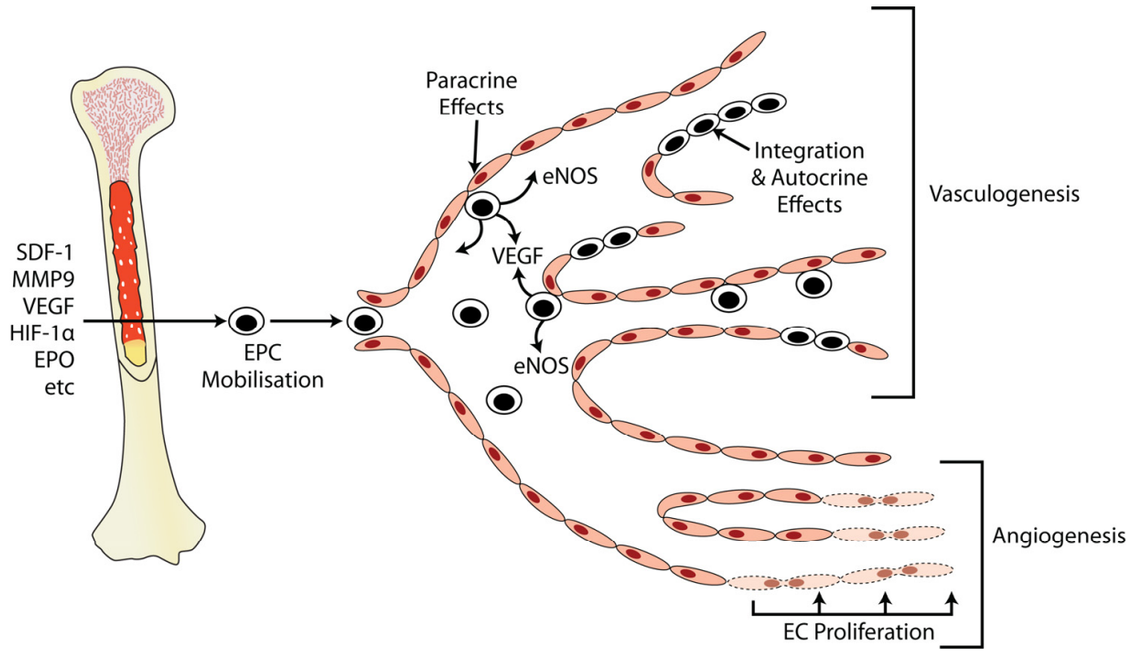
undergo apoptosis in early SSc, whereas vascular smooth-muscle cells and pericytes proliferate vigorously. This vascular damage, which eventually occurs in almost all organs (Harrison et al., 1993), presents as large gaps between endothelial cells, loss of integrity of the endothelial lining, and the formation of vacuoles in the endothelial cell cytoplasm. In addition, several basal lamina-like layers build up, mononuclear immune cells infiltrate the vessel walls, obliterative microvascular lesions occur, and the capillaries rarefy (Prescott et al., 1992, Fleming et al., 2008). In the later stages of SSc, relatively few small blood vessels remain. Serum levels of VEGF are high in SSc despite the progressive loss of blood vessels (Distler et al., 2004, Davies et al., 2006), possibly as a result of an adaptive response to hypoxia (Fleming et al., 2008, Kuwana et al., 2004, Cipriani et al., 2007). The molecular mechanisms underlying this defect in vasculogenesis are unknown and both vasculogenic (Davies et al., 2006, Distler et al., 2004) and anti-vasculogenic (Fleming et al., 2008, Hebbar et al., 2000, Scheja et al., 2000) factors have been detected in early SSc. Inflammatory cytokines, such as tumour necrosis factor (TNF), can stimulate or inhibit angiogenesis depending on the duration of the stimulus (Sainson et al., 2008). Collectively, these data indicate that vascular dysfunction is a common event in SSc and an important therapeutic target.

3. Endothelial progenitor cells

EPCs were first discovered in peripheral blood by Asahara and colleagues in 1997 (Asahara et al., 1997). This discovery revealed that vasculogenesis occurs after post-natal development. Vascular insult or disease causes the upregulation of cytokines such as VEGF, stromal cell-derived factor-1 (SDF-1) matrix metalloproteinase 9 (MMP9), hypoxia inducible factor 1α (HIF-1α) and erythropoietin (EPO) at the site of injury and this stimulates the release of EPCs from the stem cell niche in the bone marrow into the circulation (Aicher et al., 2005). EPCs then follow the cytokine gradient to the site of vascular trauma where they contribute to vasculogenesis either by (1) paracrine assistance (via production of VEGF and endothelial nitric oxide synthase (eNOS)) (2) integration or (3) new vessel formation (Figure 2).

There are currently two distinct ways in which EPCs are identified, i.e. (1) they are directly identified in the peripheral blood by the surface antigen expression of any combination of CD133, CD34 and VEGR2 or (2) they are isolated from either peripheral blood (Asahara et al., 1997), umbilical cord blood (Asahara et al., 1997, Shi et al., 1998) or bone marrow (Shi et al., 1998) and cultured ex vivo. The complication associated with using the cell surface markers CD133, CD34 and VEGFR2 to identify EPCs is that these markers are not exclusively expressed on EPCs and can be found on many other cell types including the closely related haematopoietic progenitors and mature endothelial cells as well as fibroblasts, epithelial cells and cancer stem cells (Hirschi et al., 2008, Kumar and Caplice, 2010). Further evidence of a need to standardise the isolation technique, culture conditions and phenotyping strategy is exemplified by Case et al., who suggest that it is not possible to culture EPCs from a CD133+ CD34+ VEGFR2+-sorted population (Case et al., 2007).

Currently, the term 'EPC' is used to describe two populations of cells cultured in vitro, both of which show vascular potential, but differ in both phenotype and function. The first EPC population to be characterised in vitro were the early-outgrowth EPCs, or colony forming unit-endothelial cells (CFU-ECs). Early-outgrowth EPCs form colonies after 3-5 days in culture on fibronectin-coated wells, consist of multiple thin, flat cells emanating from a



Recruitment of endothelial cells from pre-existing vessel walls or circulating endothelial progenitor cells (EPCs) play a critical role in blood vessel development and repair during disease states. Mobilised bone-marrow derived EPCs with high proliferative capacity may have the potential to home to a site for vascularisation and act in a paracrine or autocrine way to promote vessel wall development.
Abbreviations: SDF-1, stromal derived factor -1; MMP9, matrix metalloproteinase 9; VEGF, vascular endothelial growth factor; HIF-1α, hypoxia inducible factor 1α; EPO, erythropoietin; eNOS, endothelial nitric oxide.

Fig. 2. Model of postnatal angiogenesis and vasculogenesis.

central cluster of round cells and express CD133, VEGFR2 and CD34 (Hur et al., 2004). Early-outgrowth EPCs secrete pro-angiogenic factors (Hur et al., 2004, Rehman et al., 2003, Yoon et al., 2005), but are not able to form tubes when seeded alone in Matrigel (Rehman et al., 2003, Timmermans et al., 2007, Yoder et al., 2007, Yoon et al., 2005). When transplanted into mice, they are able to increase capillary density in a model of limb ischemia (Hur et al., 2004, Yoon et al., 2005), suggesting that they contribute to tube formation through paracrine mechanisms. Early-outgrowth EPCs express the pan-leukocyte marker CD45 and the myeloid marker CD14 and have been shown to be of monocyte origin (Medina et al., 2010) and are thus not considered true endothelial cell progeny.

The second EPC population to be characterised are the late-outgrowth EPCs, which as also referred to as outgrowth endothelial cells (OECs) and endothelial colony forming cells (ECFCs). Late-outgrowth EPCs can be isolated from bone marrow, cord blood and peripheral blood and form colonies with distinct cobblestone morphology, similar to that of endothelial cells within 2-4 weeks when cultured on either collagen or gelatin (Lin et al., 2000, Shi et al., 1998). Late-outgrowth EPCs have 10 times the proliferative capacity of mature ECs, they express mature endothelial cell markers including von Willebrand factor (vWF), CD31 and VEGFR2, but not the progenitor marker CD133 and they are able to form tubes in Matrigel (Bompais et al., 2004, Ingram et al., 2004, Lin et al., 2000, Rehman et al., 2003, Timmermans et al., 2007, Yoder et al., 2007, Yoon et al., 2005). Late-outgrowth EPCs

have been shown to increase neovascularisation in a mouse limb ischemic model (Hur et al., 2004, Yoon et al., 2005) and are haemangioblastic in origin (Medina et al., 2010) and are thus considered to be true endothelial cell progeny.
Whilst the monocytic early-outgrowth EPCs and the haemangioblastic late-outgrowth EPCs are distinct EPC populations, the combined therapeutic potential of these two EPC populations is greater than either of the EPC populations when delivered individually in a mouse model of limb ischemia (Yoon et al., 2005), suggesting that these EPC populations may function synergistically during vasculogenesis.

4. Endothelial progenitor cells in autoimmune disease

4.1 Endothelial progenitor cells in rheumatoid arthritis

The association between EPC numbers and RA has brought about conflicting results (Table 1). Some studies have reported a lower circulating EPC number in RA patients compared with controls (Grisar et al., 2005, Herbrig et al., 2006), whilst others report higher numbers (Jodon de Villeroche et al., 2010) and a few report no differences (Egan et al., 2008, Kuwana et al., 2004). A schematic of a potential role for EPCs in RA is depicted in Figure 1 (upper panel).
In the studies that reported lower circulating EPC numbers in patients with active RA compared to healthy controls (Grisar et al., 2005, Herbrig et al., 2006), the circulating EPCs were identified through the expression of CD133, CD34 and VEGFR2 and the formation of early-outgrowth EPC colonies. It is highly likely that these studies were not specifically identifying a pure EPC population, but rather a mixed population consisting of both early-outgrowth EPCs, late-outgrowth EPCs and haematopoietic progenitors, as the biomarkers used to identify EPCs are not specific for any one cell type.
In contrast, Jodan de Villeroche et al used a method to exclusively identify haemangioblastic late-outgrowth EPCs distinct from monocytic early-outgrowth EPCs (Jodon de Villeroche et al., 2010). Jodan de Villeroche et al exclusively monitored the number of late-outgrowth EPCs by detecting Lin-/7-aminoactinomycin (7-AAD)-/CD34+/CD133+/VEGFR2+ cells from CD14 depleted peripheral blood. This detection panel eliminated apoptotic cells (using 7-AAD) and early-outgrowth EPCs (through CD14 depletion). Using these methods this study revealed that RA patients with active RA had significantly higher levels of circulating late-outgrowth EPCs compared with controls. To complement these findings, this study also investigated the formation of late-outgrowth EPC colonies and found that RA patients had a higher number of late-outgrowth colonies compared to controls. This study was the first to implement a method that made a distinction between the two EPC populations.

4.2 Endothelial progenitor cells in type 1 diabetes mellitus

A decrease in EPC number and function has been associated with T1DM and has been reported by several groups (Table 2). However before comparisons can be made between studies, it is important to consider the methods used to quantify EPC numbers in these studies. Circulating EPC numbers were quantified either by surface antigen expression on peripheral blood mononuclear cells (PBMNCs) (Brunner et al., 2009, Sibal et al., 2009), through the culture of early-outgrowth EPC colonies (Asnaghi et al., 2006) or through the uptake of acetylated LDL and binding of UEA-l to cultured PBMNCs (Loomans et al., 2004). To the best of our knowledge, there are currently no reports on the correlation between T1DM and the growth of late-outgrowth EPCs.

Reference	Method of EPC identification	Comments
Grisar et al., 2005	Expression of CD133/CD34/VEGFR2 from PBMNC using flow cytometry. In vitro culture of PBMNCs and detection of early-outgrowth EPCs colonies.	EPCs were lower in active RA patients compared to healthy controls when assessing surface antigen expression. Reduced number of early-outgrowth EPC colonies in active RA patients compared to healthy controls.
Grisar et al., 2007	Expression of CD133/CD34/VEGFR2 from PBMNC using flow cytometry.	TNF may be partly responsible for the reduction of circulating EPCs seen in RA patients.
Egan et al., 2008	Expression of CD133/CD117/CD34/CD31 from PBMNCs using flow cytometry. In vitro culture of PBMNCs and detection of early-outgrowth EPCs colonies.	No difference in the number of EPCs in RA patients and healthy controls when assessing surface antigen expression. Reduced number of early-outgrowth EPCs in active RA patients compared to healthy controls. Early-outgrowth EPC colony numbers were associated with cardiovascular risk.
Jodon de Villeroche et al., 2010	Surface antigen profile Lin-/7AAD-/CD34+/CD133+/VEGFR2+ from CD14-depleted PBMNCs using flow cytometry. In vitro culture PBMNCs and detection of late-outgrowth colonies.	RA patients had higher numbers of circulating EPCs than healthy controls. Circulating EPCs correlated with disease activity.
Herbrig et al., 2006	In vitro culture of PMNCs and assessment of Ac-LDL uptake, UEA-1 lectin binding and the surface antigen profile VE-cadherin+/CD31+/VEGFR2+/CD146-.	EPCs from RA patients showed reduced migratory activity in response to VEGF.

Abbreviations: RA, rheumatoid arthritis; PBMNCs, peripheral blood mononuclear cells; EPCs, endothelial progenitor cells; Ac-LDL, acetylated-low density lipoprotein; UEA-1 lectin, *Ulex Europaeus* Lectin; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Table 1. Studies that have reported aberrant EPC numbers in patients with RA.

EPC dysfunction has been seen in patients with T1DM, as shown by Loomans et al. when conditioned media from EPCs isolated from T1DM patients impaired in vitro tube formation of HUVEC (Loomans et al., 2004). An inverse relationship between the number of

EPCs and HbA1C in patients has also been identified (Loomans et al., 2004). Moreover, there appears to be an association between the progression of diabetic retinopathy and the level of circulating EPCs. In patients with T1DM and proliferative retinopathy a marked increase in circulating EPCs has been reported (Asnaghi et al., 2006, Brunner et al., 2009). Conversely, circulating EPC numbers have been identified as being lower in patients with T1DM and non-proliferative retinopathy (Brunner et al., 2009). These studies highlight how atypical EPC numbers and function are associated with T1DM pathology and a schematic of a potential role for EPCs in diabetic retinopathy is depicted in Figure 1 (middle panel).

Reference	**Method of EPC identification**	**Comments**
Loomans et al., 2004	In vitro culture of PMNCs and assessment of Ac-LDL uptake, UEA-1 lectin binding and CD31 expression.	T1DM patients had lower EPC levels compared to healthy controls.
Sibal et al., 2009	Expression of CD133/CD34/VEGFR2/VE-cadherin from PBMNC using flow cytometry.	EPC counts were lower in patients with T1DM compared to healthy controls.
Asnaghi et al., 2006	Immunostaining with CD133 and CD31	Patients with T1DM and retinopathy had higher EPC levels than healthy controls and patients with T1DM and no retinopathy.
	In vitro culture of PBMNCs and detection of early-outgrowth EPCs colonies.	Patients with T1DM and no retinopathy had lower EPC levels than healthy controls and patients with T1DM and retinopathy.
Brunner et al., 2008	CPC surface antigen profile CD133+/CD34+	Patients with T1DM and proliferative retinopathy had increased levels of mature EPCs.
	EPCs surface antigen profile CD133+/CD34+/VEGFR2+	Patients with T1DM and nonproliferative retinopathy had decreased levels of EPCs.
	Mature surface antigen profile CD133+/CD34+/VEGFR2+/CD31+	
	Nonmature surface antigen profile CD133+/CD34+/VEGFR2+/CD31-	

Abbreviations: T1DM, type 1 diabetes mellitus; PBMNCs, peripheral blood mononuclear cells; EPCs, endothelial progenitor cells; CPC, circulating progenitor cells; Ac-LDL, acetylated-low density lipoprotein; UEA-1 lectin, *Ulex Europaeus* Lectin

Table 2. Studies that have reported aberrant EPC numbers in patients with T1DM.

4.3 Endothelial progenitor cells in systemic sclerosis

Aberrant EPC numbers within the circulation of patients with SSc has been described extensively (Table 3). The majority of these studies used flow cytometry to assess EPC numbers using various combinations of the markers CD133, CD34 and VEGFR2. As mentioned previously, the use of these markers does not unambiguously identify circulating EPCs as they are expressed by other progenitor cells and mature endothelial cells. Avouac et al describe the most stringent method of EPC identification, which involved culturing the PBMNCs from both SSc patients and healthy controls and assessing late-outgrowth EPC colony formation. This study showed that the number of late-outgrowth EPC colonies correlated with the number of circulating EPCs detected using the surface antigen profile Lin-/7AAD-/CD34+/CD133+/VEGFR2+ (Avouac et al., 2008).

Reference	Method of EPC identification	Comments
Allanore et al., 2007	Expression of CD133/CD34 from PBMNC using flow cytometry.	SSc patients had higher numbers of EPCs than osteoarthritis patients, but lower than RA patients.
Yamaguchi et al., 2010	In vitro culture of PBMNCs depleted for platelets. Expression of CD34/VEGFR1/CD1a/CD83/CD80 using flow cytometry and CD31/CD144 by immunohistochemistry.	The number of early-outgrowth EPCs was higher in SSc patients compared to RA patients and healthy controls. Early-outgrowth EPCs derived from SSc patients showed greater vascular potential in vitro and in vivo than early-outgrowth EPCs derived from healthy controls.
Kuwana et al., 2004	Expression of CD133/CD34/VEGR2 from CD34-enriched PBMNC using flow cytometry.	EPCs were lower in SSc patients compared to RA patients and healthy controls. Levels of angiogenic factors within the circulation were higher in SSc patients than in health controls.
Kuwana et al., 2006	Expression of CD133/CD34/VEGR2 from CD34-enriched PBMNC using flow cytometry.	Atorvastatin treatment resulted in an increase in circulating EPCs from baseline, however levels did not reach those of healthy controls.
Del Papa et al., 2004	Surface antigen profile CD133+/CD34+ from PBMNC using flow cytometry.	High levels of EPCs in patients with SSc and counts were higher in early stages of disease.
Del Papa et al., 2006	Surface antigen profile CD133+/CD45- from PBMNC and BM using flow cytometry.	Circulating EPCs were higher in patients with early stage disease, but not in those with late stage disease. BM EPCs were reduced and functionally impaired.
Avouac et al., 2008	Surface antigen profile Lin-/7AAD-/CD34+/CD133+/VEGFR2+ from PBMNCs detected using flow cytometry. In vitro culture of PBMNCs and detection of late-outgrowth colonies.	Circulating EPC levels were higher in SSc patients than in healthy controls. Positive correlation between the number of late-outgrowth EPC colonies and the level of circulating EPCs detected by flow cytometry in patients with SSc.

Abbreviations: SSc, systemic sclerosis; PBMNCs, peripheral blood mononuclear cells; EPCs, endothelial progenitor cells; BM, bone marrow.

Table 3. Studies that have reported aberrant EPC numbers in patients with SSc.

It has been well documented that circulating EPC numbers are elevated in patients with SSc (Allanore et al., 2007, Avouac et al., 2008, Del Papa et al., 2004, Del Papa et al., 2006, Yamaguchi et al., 2010). However, two studies by Kuwana and colleagues have reported reduced EPC numbers in SSc patients (Kuwana et al., 2006, Kuwana et al., 2004). Del Papa et al showed that in early stage SSc (3-5 years) there appears to be an increase in circulating EPCs and post 5 years, there appears to be either a normal or decreased number of circulating EPCs (Del Papa et al., 2006). A schematic of a potential role for EPCs in SSc is depicted in Figure 1 (lower panel). There is also evidence to suggest that the vascular function of EPCs from SSc patients is actually higher than that of healthy controls as early-outgrowth EPCs from SSc patients are able to promote tube formation of HUVEC in vitro as well as enhance tumour growth and blood vessel formation in vivo (Yamaguchi et al., 2010).

5. Therapeutic intervention targeting EPCs in autoimmune diseases

There have been rapid advances in the field of therapeutic angiogenesis since the original description of bone marrow derived EPCs in 1997 (Asahara et al., 1997). Most of these bench-to-bed-side studies have been done in models of atherosclerosis and acute ischemic events such as myocardial infarction (MI) and critical limb ischemia. The first pre-clinical studies in these diseases were executed (within four years of their initial discovery) in a MI model in mice (Kocher et al., 2001) and demonstrated improvement in angiogenesis and cardiac function. This was followed by a series of publications showing the effectiveness of EPCs in preventing the extent of damage (Orlic et al., 2001) after MI as well as effectiveness in the large vessel occlusive damage (Griese et al., 2003) and prevention of atherosclerosis in a highly prone mouse model (Rausher et al, 2003). However, the exact mechanism of action of these interventions, in particular whether the benefit was due to neo-angiogenesis modulated by EPCs or due to paracrine mechanisms that improved the survival of resident endothelial cells, is not entirely clear.

There was a rapid transition of these studies to humans as autologous marrow transplantation became a relatively safe and well established procedure in haematological malignancies and non-invasive methods to mobilise bone marrow progenitors became well established. In 2002, there were two studies published reporting the benefit of locally injecting ex-vivo expanded autologous bone marrow derived mononuclear cells in MI critical lower limb ischemia (Strauer et al., 2002, Tateishi-Yuyama et al., 2002). Furthermore, there have been multiple randomised controlled trials looking at the effectiveness of bone marrow derived cell therapies, which have been reviewed in a recent meta-analysis (Martin-Rendon et al., 2008).

The therapeutic use of EPCs in inflammatory diseases is more complicated as they have been implicated in pathogenesis of the inflammatory process as well as being an important cause of long term morbidity. There have been no studies of direct intervention with EPCs in autoimmune diseases. This is mainly due to their differential effects on the immunopathogenesis of these diseases. Attempts to understand this field are further bedevilled by observations of patients with systemic lupus erythematosus (SLE) who exhibit a significant decrease in circulating EPCs as well as a striking increase in premature atherosclerosis of unclear aetiology (de Leeuw et al., 2005, Westerweel et al., 2007) demonstrating no significant difference in EPC number between SLE patients without and with advanced coronary artery calcification (Baker et al., 2011). As detailed above, the inflammatory milieu in autoimmune diseases is characterized by neo-angiogenesis and as

such it would seem that increased EPCs might contribute to inflammation. On the other hand, the most common cause of long term morbidity and mortality in these diseases is attributed to atherosclerosis and its complications where EPCs might have beneficial effect. There have been numerous studies looking at the effect of various disease modifying therapies in patients with autoimmune diseases on the circulating EPCs (both monocytic and haemangioblastic) reviewed in a recent article (Westerweel and Verhaar, 2009). These studies show an increase in the levels of circulating haemangioblastic EPCs after various immunosuppressive therapies including anti-TNF drugs, corticosteroids and hydroxychloroquine in these patients. However, the association of these changes with long term clinically useful outcome, such as incidence of atherosclerosis and coronary artery disease, has not been demonstrated. This finding is intriguing as it is well known from long term clinical studies that corticosteroids are known to promote atherosclerosis and anti-TNF medications reduce long term morbidity and mortality due to this complication (Kaplan, 2010). Moreover, methotrexate, a commonly used disease modifying agent in various autoimmune diseases is known to induce EPC apoptosis in vitro (Herbrig et al., 2006) but has beneficial effects in patients.

There needs to be better understanding of the role of EPCs in the different stages of the disease, i.e. early active versus long standing, and its pathophysiological implications relating to long term outcome of patients to be able to design studies with intervention directed at EPCs. The knowledge of various paracrine mechanisms involved in the beneficial effects of EPCs in atherosclerosis models might help to dissect the pathways involved in neo-angiogenesis versus survival of resident endothelial cells. This knowledge can then be exploited to design intervention at various stages of autoimmune diseases.

6. Conclusions

Faced with an ever-increasing burden of autoimmune diseases such as RA, T1DM and SSc, modern medicine is confronted with the need to provide new therapies that not only mitigate the symptoms of these diseases but may also facilitate regeneration of organ function. Given their role in development and in maintaining and repairing injured vessels, stem and progenitor cells represent an exciting alternative for regenerative medicine. Since their first identification over a decade ago, the use of EPCs as a diagnostic tool or therapeutic was greeted with great enthusiasm. However, progress in their clinical application remains limited by identification and *ex vivo* expansion factors, and as a result, variable functional attributes. It can be seen from the aforementioned examples that the timing and methods used to detect EPCs can greatly affect the outcome of studies. The major issues associated with EPC identification within the circulation are (1) identifying the bone marrow progenitors from the circulating mature endothelial cells and (2) defining the distinction between haemangioblastic late-outgrowth EPCs and monocytic early-outgrowth EPCs. These matters are the focus of ongoing research, especially the search for a unique EPC marker. Nevertheless, EPCs are a robust biomarker of vascular dysfunction (based on their direct interaction and influence on endothelial function), and the unique ability to monitor their peripheral number or function as a marker of response to therapy. Notwithstanding the current knowledge regarding EPC cell signalling, activation and migration, the precise mechanisms of activation of these cells and their functional significance is not known. In the research setting, continued understanding of EPC function improves insight into vasculogenesis, and the pathology of vascular dysfunction in autoimmune disease.

7. Acknowledgements

This work was supported by the Co-operative Research Centre for Biomarker Translation (Transbio Ltd) and Arthritis Australia. CSB is a Heart Foundation Research Fellow and we thank P. Dunne for preparation of the figures.

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