Endothelial Progenitor Cells
Novel Players in the Pathogenesis of Rheumatic Diseases

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Introduction

Cardiovascular disease is an integral part of most rheumatic diseases, and its impact on the outcome and prognosis of the rheumatic disease is a major research focus for rheumatologists and cardiologists. In patients with inflammatory rheumatic disorders, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), the cardiovascular risk is highly increased, even if other cardiovascular risk factors known to promote and accelerate the progression of atherosclerotic lesions are absent. Patients with RA have a 2–5-fold increased risk of developing premature cardiovascular disease. The increased risk of myocardial infarction and stroke strongly contributes to the increased mortality risk and to the shortened (by 5–10 years) life expectancy of patients with RA (1,2).

Other rheumatic disorders, such as systemic sclerosis (SSc), are characterized by a severe vasculopathy. Involvement of the microvascular circulation manifests as Raynaud's syndrome and ischemic ulcers, and it has a profound impact on the quality of life (3,4). In addition, involvement of the pulmonary vasculature leads to pulmonary arterial hypertension (PAH) in 10–20% of patients with SSc. PAH is also known to be a major cause of death in SSc (5,6). As illustrated by these examples, rheumatic disorders are characterized by a profound cardiovascular disease and may therefore even serve as research models for studying the influence of systemic inflammatory processes on cardiovascular integrity.

Over the last 13 years, endothelial progenitor cells (EPCs) have emerged as crucial regulators of cardiovascular integrity. Reduced numbers and altered functions of EPCs have been found to be involved in the pathogenesis of cardiovascular disease. The important role of vasculogenesis and EPCs in adults was discovered late. The formation of new vessels had long been believed to be mediated exclusively by the sprouting of fully differentiated endothelial cells (ECs) from preexisting vessels, a process called angiogenesis. This concept was first disproved by Asahara and colleagues (7) in 1997. In a landmark study, they demonstrated that new blood vessels can also be formed in adults by circulating progenitor cells, independently of the preexisting vasculature (7). They found that bone marrow–derived CD34+ cells could acquire the characteristics of mature ECs, express endothelial markers, and incorporate into new vessels at sites of ischemia.

Subsequent analyses revealed that these EPCs were not restricted to the bone marrow, but could also be detected in the peripheral circulation of adults (8). Since then, growing evidence has suggested that EPCs play an important role in the homeostasis of the physiologic vascular network. EPCs are not only involved in the formation of new vessels in ischemic tissues, thereby contributing to the vascular remodeling, but might also...
contribute to the repair of existing vessels (8–10). This concept is indicated by the inverse correlation of circulating EPC numbers with cardiovascular risk factors, and it is further supported by the observation that conditions which reduce the cardiovascular risk increase the levels of EPCs (11–15).

In addition to the defective EPC compartment observed in patients with primary cardiovascular diseases, reduced numbers and/or altered functions of EPCs have recently been demonstrated in patients with SSc (16–23), RA (24–30), SLE (31–34), and vasculitides (35). In SSc, capillary rarification and vascular alterations might be partly due to the impaired de novo formation of new blood vessels by EPCs. Vasculopathy in SSc is known to cause ischemic manifestations, such as fingertip ulcers and gangrene. In patients with RA or SLE, the defective vasculogenesis is likely to contribute to the increased cardiovascular risk. Further studies on vasculogenesis and EPCs may help us to understand vasculopathy and defective vascular repair mechanisms, which are major pathogenic characteristics of many rheumatic disorders.

New insights into the role of EPCs in the vasculopathy of rheumatologic disorders might be of interest for several reasons. First, alterations in the numbers of circulating EPCs and their functional impairments have been demonstrated in several rheumatic disorders and may therefore be relevant to pathogenic processes (16–35). Decreased EPC counts and impaired EPC functions, which result in impaired vasculogenesis, might explain, at least in part, the often characteristic widespread vasculopathy. Second, EPCs might be sensitive biomarkers of cardiovascular involvement in rheumatic diseases, and decreased EPC counts might become established as novel markers of increased cardiovascular risk. In inflammatory vasculitides, EPCs have already been shown to be inversely correlated with disease activity scores (13). Third, EPCs offer a broad therapeutic potential. Application of autologous in vitro–amplified EPCs has been shown to stimulate vessel formation and to improve the outcome of patients with myocardial infarction or mice with hind limb ischemia (36,37). Therefore, injection of EPCs might be a promising approach for the treatment of patients with SSc, who have ischemic fingertip ulcers and gangrene due to the progressive loss of capillaries. Alternatively, EPCs might be used as vehicles for the transport of drugs to activated endothelium and hypoxic tissues. Since EPCs home to sites of ischemic tissue injury, this approach would allow targeted therapy of involved organs.

Taken together, there is a rapidly growing interest in elucidating the role of EPCs in the pathogenesis of rheumatic disorders and in developing novel diagnostic and therapeutic applications. This review summarizes the current knowledge about EPCs in rheumatic diseases and discusses open issues about different EPC subsets, their mode of action, and their relevance in vascular repair and integrity, which need to be addressed in further studies.

**Subpopulations of EPCs**

Two main subpopulations of EPCs with different origins, morphologic characteristics, and functions have been identified. These 2 subpopulations can be differentiated by the expression of the cell surface marker CD14. Short-term cultures of ≤7 days mainly contain EPCs of the CD14+ subpopulation. This subset of EPCs is thought to represent transdifferentiated CD14+ monocytes, which acquire characteristics of ECs under certain culture conditions (38). In vitro, CD14+ EPCs show little proliferation capacity and undergo apoptosis within a few weeks (39,40). In contrast to the CD14+ EPCs arising from short-term cultures, CD14− EPCs are harvested from long-term, late-outgrowth cultures. The CD14− subset of EPCs, which sometimes is referred to as “true EPCs” or “angioblast-like EPCs,” possess an extraordinarily high proliferation capacity (41).

Besides differentiation according to the CD14 cell surface marker, the 2 EPC subsets can also be distinguished morphologically. CD14+ EPCs are spindle-shaped, whereas CD14− EPCs form cobblestone monolayers (38,39). Interestingly, both CD14+ EPCs and CD14− EPCs can form capillary tubes in vitro, mediate reendothelialization after vessel injury, and improve neovascularization (9,42). Similar to CD14– EPCs, CD14+ EPCs can be incorporated into vessels after short-term culture under conditions that promote their differentiation into EPCs. In contrast, CD14+ cells without ex vivo differentiation do not promote neovascularization (43).

Based on these observations and considering their different origins, specific functions have been proposed for CD14+ and CD14− EPCs (9,10). CD14+ EPCs are supposed to stimulate the formation of granulation tissue and new vessels by releasing mediators of inflammation as well as vascular growth factors, whereas their integration into vessels is of minor importance. In contrast, CD14− angioblast-like EPCs might strongly proliferate and differentiate at sites of vascular injury after integration into the vessel wall. Proliferation and differentiation of EPCs might result in a pool of ECs,
which promotes and orchestrates vessel repair and the local formation of new vessels via secretion of proangiogenic factors.

Aside from the CD14+ and CD14− subsets of EPCs, there is evidence that mesenchymal stem cells and tissue-resident stem cells might also differentiate into EPCs. However, their physiologic relevance is still unclear (44–46).

**Mobilization of EPCs from the bone marrow**

The mobilization of EPCs from the bone marrow and their release into the peripheral circulation rely on a complex cascade of signaling events. The translocation of early c-Kit+ progenitor cells prompts the release of EPCs from the quiescent bone marrow stromal niche into the vascular zone of the bone marrow. The migration of c-Kit+ progenitor cells is initiated by cleavage of membrane-bound c-Kit ligand to its soluble form by matrix metalloproteinase 9 (MMP-9) (47). The expression of endothelial cell nitric oxide synthase (eNOS) by local stromal cells seems to be required for the release of EPCs from the bone marrow into the circulation (48). Mice deficient in eNOS showed impaired neovascularization due to the diminished mobilization of EPCs. The defects in neovascularization in mice lacking eNOS were rescued by infusion of wild-type EPCs, but not by bone marrow transplantation. On the molecular level, eNOS may contribute to the activation of MMP-9 (48).

Differentiation of primitive c-Kit+ cells leads to the formation of bone marrow hemangioblasts, which are early progenitor cells of vascular and hematopoietic cells (49). Activation of vascular endothelial growth factor receptor 2 (VEGFR-2) by VEGF is crucial for further maturation of hemangioblasts and EPCs (50,51). Besides VEGF, other cytokines are also important for the maturation and mobilization of EPCs. Granulocyte-colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF) mobilize CD34+ cells, including EPCs, in the bone marrow (52). Stem cell–derived factor 1 (SDF-1) induces a rapid increase in the number of circulating EPCs similar to VEGF165, whereas the mobilization of EPCs by angiopoietin 1 is delayed (53).

**Neovascularization and reendothelialization by EPCs**

Numerous studies have demonstrated that EPCs contribute to vascular homeostasis by inducing neovascularization in ischemic tissues and by stimulating reendothelialization after vascular injury. EPCs from different sources isolated by various protocols have been shown to increase the capillary density and improve neovascularization at sites of ischemia. Kocher and coworkers (36) were the first to demonstrate that infusion of CD34+ cells that had been isolated from the peripheral blood stimulated the de novo formation of vessels and the spreading of new vessels from the preexisting vasculature in experimental myocardial infarction. Apoptosis of myocytes in the peri-infarct area was reduced in treated animals, scarring of the myocardial tissue was diminished, and cardiac function was improved.

At the same time, Kawamoto et al (37) showed that in vitro–differentiated, early-outgrowth EPCs accumulated at sites of ischemia and incorporated into foci of neovascularization in experimental myocardial infarction. EPCs were also found to improve neovascularization in experimental models of hind limb ischemia. After induction of hind limb ischemia, infusion of ex vivo–expanded EPCs into athymic mice or rats increased the capillary density and enhanced the local blood flow, resulting in a reduced rate of limb loss (54,55). Of note, infusion of mature ECs did not improve vascularization in these models (54,56).

Besides their beneficial effects in the experimental models of myocardial infarction and hind limb ischemia, EPCs also stimulated reendothelialization in a rat model of balloon injury of the carotid artery and prevented neointimal thickening (15). Furthermore, reendothelialization of vascular grafts has also been shown to be mediated by EPCs (41). EPCs derived from the peripheral blood and expanded in vitro formed confluent monolayers on decellularized porcine iliac vessels. EPC-seeded grafts exhibited nitric oxide–mediated vascular relaxation and contractile activity comparable to that of native carotid arteries. These grafts remained patent throughout the observation period of 130 days, whereas nonseeded grafts occluded rapidly.

The encouraging results with EPCs in experimental models of tissue ischemia led to the initiation of clinical trials in humans. Tateishi-Yuyama and coworkers (57) demonstrated that autologous transplantation of mononuclear cells (MNCs), which contain EPCs among other MNC populations, improved critical limb ischemia. Intramuscular injections of bone marrow–derived MNCs into the calf significantly increased the oxygen pressure, ankle–brachial index, and walking distance as compared with saline-treated control patients. In addition, the authors demonstrated that MNCs derived from the peripheral blood were significantly less effective than bone marrow–derived MNCs. The num-
ber of CD34+ cells, including EPCs, is ~500-fold higher in bone marrow than in peripheral blood, a fact which might explain the markedly enhanced efficacy of the transplantation of bone marrow–derived MNCs. Tateishi-Yuyama et al (57) did not study whether the injected MNCs integrated into the vessels after intramuscular injection. However, expression of the proliferation marker Ki-67 was found to be increased in ECs, which might have been caused by the release of growth factors, such as VEGF, angiopoietin 1, basic fibroblast growth factor, tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and IL-6 from the injected cells.

Several randomized controlled clinical trials have documented the efficacy of intracoronary injections of blood-derived or bone marrow–derived EPCs in the treatment of acute myocardial infarction (58–61). Patients receiving EPCs had presented with enhanced tissue perfusion, higher ejection fractions, and increased wall motion. Of note, one trial was stopped because of a high rate of in-stent stenoses. Although increased in-stent restenosis was not reported in the other trials, the infusion of EPCs might potentially lead to uncontrolled neovascularization at unwanted tissue sites (9). Uncontrolled neovascularization could lead to increased vascularization of atherosclerotic plaques, thereby contributing to growth and destabilization of the plaques.

Because of their proliferative and stimulatory nature, EPCs might promote carcinogenesis, a serious side effect that is difficult to study in humans. In models of tumor angiogenesis, however, EPCs stimulate the formation of new tumor vessels (62,63). Of note, the number of cells originating from EPCs in the vessel wall tended to be higher in models of tumors than in models of ischemia, suggesting a critical role of EPCs in tumor vascularization.

**Molecular mechanisms involved in the neovascularization by EPCs**

Neovascularization and reendothelialization by EPCs are complex multistep processes, requiring the chemoattraction of EPCs to sites of tissue ischemia or vascular injury, adhesion of EPCs, and their differentiation into mature ECs. The signaling cascades that regulate these steps are still incompletely understood and are the focus of intensive research. However, several important pathways have been uncovered during the last several years.

**Chemokines and cytokines.** Early studies on mice deficient in VEGF, VEGFR-1, and VEGFR-2 demonstrated the essential role of VEGF signaling in embryonic vasculogenesis (64–66). Similar to its importance during embryonic development, VEGF and its receptors are also critical for vasculogenesis in adults. VEGF mobilizes EPCs within the bone marrow and induces the differentiation of EPCs into ECs in ex vivo culture assays (54). The chemokine CXCL12 (SDF-1) also acts as a chemoattractive factor for EPCs and increases the release of EPCs from the bone marrow to the blood (51,67–69). VEGF and CXCL12 (SDF-1) are oxygen-sensitive cytokines that are induced by hypoxia (70). VEGF and CXCL12 act as molecular mediators to mobilize EPCs from the bone marrow and to guide them into ischemic tissues (51,67–69).

Ligands of CXCR2 might also direct EPCs to sites of vascular repair and neoangiogenesis and increase the adhesion of EPCs. EPCs express CXCR2, and incubation of EPCs with CXCR2 ligands such as CXCL1 (growth-related oncogene α) and CXCL7 (neutrophil-activating peptide 2) increases adhesion of EPCs to platelet-coated endothelial matrices in vitro and to sites of arterial injury in vivo (71). Further analysis showed that CXCR2+ EPCs strongly adhered to injured arteries and that the CXCR2+ EPCs predominantly expressed the monocytic marker CD14 (71). A number of CC chemokines have been implicated in EPC homing. Using a transgenic mouse model of multistep carcinogenesis, Spring et al (72) demonstrated that ECs isolated from tumors released CCL2 (monocyte chemotactic protein 1), CCL3 (macrophage inflammatory protein 1α), and CCL5 (RANTES), which directed a subset of CCR2+ and CCR5+ EPCs into the tumor vasculature (72). Inhibition of chemokine receptor signaling by the nontoxic inhibitor pentoxifylline strongly decreased the integration of EPCs into vessels, confirming the functional relevance of CC chemokines in the integration of EPCs into the tumor vasculature (72).

In addition, proinflammatory cytokines, including TNFα, IL-1β, and IL-6, which are released by infiltrating leukocytes, might attract CD14+ progenitor cells, increase their adherence to the injured endothelium by a β1 integrin–dependent mechanism, and stimulate their differentiation into mature ECs (73).

**Adhesion molecules.** Adhesion of EPCs to decellularized vessels might be mediated by adhesion to vitronectin via αvβ3 and αvβ5 integrins, since reendothelialization in vivo was inhibited by the blocking of αvβ3 and αvβ5 integrins with cyclic arginine-glycine-aspartic acid (RGD) peptides (74). The β2 integrin is supposed to be particularly important for the adhesion of EPCs and for transendothelial migration (75). Al-
though EPCs overexpress messenger RNA for αL, αM, αX, and β2 integrins as compared with human umbilical vein endothelial cells (HUVECs), only blocking antibodies against β2 integrin inhibit adhesion and transmigration of EPCs, and EPCs from β2 integrin–deficient mice are less capable of homing to ischemic tissues. Finally, incubation of EPCs with β2 integrin–activating antibodies was shown to stimulate neovascularization in an animal model of hind limb ischemia.

Activation of erythropoietin-producing human hepatocellular carcinoma (Eph) receptors might stimulate local adhesion of EPCs and increase their proangiogenic potential (76). Induction of EphB4 with an ephrin B2–Fc chimeric protein enhanced the adhesion of EPCs by up-regulating the expression of P-selectin glycoprotein ligand 1 (PSGL-1). Inhibition of EphB4 signaling by small interfering RNA (siRNA) or by blockade of PSGL-1 with its receptors E-selectin and P-selectin abolished the stimulatory effects of ephrin B2–Fc chimeric proteins and decreased the proangiogenic potential of EPCs in the nude mouse model of hind limb ischemia.

Matrix-degrading enzymes. In a series of experiments, Urbich et al (56) demonstrated the importance of cathepsin L for tissue invasion of EPCs during the formation of new blood vessels. Cathepsin L is expressed at higher levels in EPCs than in HUVECs, and its activity is significantly increased. Incubation of EPCs with the cathepsin L inhibitor Z-FF-FMK or with cystatin C, a general inhibitor of papain-like cysteine peptidases, reduced the invasiveness of EPCs and decreased their neovascularization ability in vivo. Similar results were obtained with EPCs from mice lacking cathepsin L. In contrast, inhibition of cathepsin S, MMPs, or elastases showed no effects on EPC function. The exceptional role of cathepsin L in the formation of new vessels can also be demonstrated by overexpression of cathepsin L in HUVECs and mature aortic endothelial cells. Infusion of mature ECs normally does not improve neovascularization. However, ectopic overexpression of cathepsin L in HUVECs or aortic endothelial cells was shown to result in the generation of cells that promoted neovascularization at sites ischemia.

Histone deacetylation and homeobox (HOX) proteins. Global histone deacetylation plays a critical role in the in vitro differentiation of embryonic stem cells (77). Differentiation of EPCs also depends on increased histone deacetylation (78). Chemical inhibitors of histone deacetylation block endothelial differentiation of EPCs without affecting adhesion or cell survival. The blocking of EPC differentiation might be mediated by the transcriptional regulator HOX-A9. The expression of HOX-A9 is increased in EPCs during endothelial differentiation, and it regulates the expression of eNOS, VEGFR-2, and probably VE-cadherin. Blockade of histone deacetylation strongly reduced the expression of HOX-A9. The importance of HOX-A9 for neovascularization was further demonstrated using HOX-A9–knockout mice, in which the number of EPCs was found to be significantly lower and EPCs were characterized by impaired postnatal neovascularization. In addition, overexpression of HOX-A9 partially reversed the reduction of EPCs, provoked by histone deacetylase inhibitors (78).

HOX-B5, another member of the HOX family of homeodomain transcription factors, also appears to be involved in the in vitro differentiation of embryonic progenitor cells toward the endothelial lineage (79). HOX-B5 regulates the expression of VEGFR-2. Therefore, overexpression of HOX-B5 in murine embryonic stem cells increased proliferation and stimulated the formation of blood vessels. Finally, the related homeobox gene HEX (or, PRH) plays a decisive role in early stages of EPC differentiation (80). HEX is preferentially expressed in hemangioblasts, the primitive progenitor cells of ECs and hematopoietic cells, and it is down-regulated during terminal differentiation into ECs. Embryonic stem cells deficient in HEX differentiation normally into hemangioblasts in vitro, but further differentiation of the HEX–/– hemangioblast into ECs, and especially into hematopoietic cells, was significantly reduced.

Vasculogenesis in the pathophysiology of SSc

Recent studies have demonstrated a role of EPCs in the pathogenesis of several rheumatic disorders, including SSc, RA, and SLE. Reduced capillary density and an irregular, chaotic architecture of the capillary network are hallmarks of SSc (3). These changes result in decreased capillary blood flow, which causes a lack of nutrients and severe tissue hypoxia in the affected organs (70). Despite the strong up-regulation of proangiogenic factors such as VEGF, sufficient vessel formation does not occur (3,22,81). It had been thought that defective angiogenesis and increased apoptosis of mature ECs were exclusively responsible for the vascular alterations in SSc; however, several studies published over the last few years have suggested that vasculogenesis might also contribute to the vasculopathy in SSc (16–23).

Kuwana et al (22) first investigated whether
vasculogenesis might be affected in patients with SSc. They defined EPCs as circulating CD34\(^+\), CD133\(^+\), and VEGFR-2\(^+\) MNCs. Using this definition, the absolute numbers of EPCs were found to be lower in patients with SSc than in patients with RA or in healthy subjects. Comparison of RA patients with healthy subjects revealed no differences in EPC counts. The numbers of EPCs were stable beyond a 3-month period in all subjects. In SSc patients, EPC counts did not correlate with the disease subset, the disease duration, or the modified Rodnan skin thickness score. However, the numbers of EPCs were lower in SSc patients with pitting scars and active fingertip ulcers. Additional in vitro experiments demonstrated an impaired differentiation capacity of EPCs isolated from the peripheral blood of SSc patients. After 5 days under standard culture conditions, only a few EPCs from patients with SSc expressed the EC marker von Willebrand factor, indicating impaired differentiation of early-outgrowth EPCs into mature ECs.

In a subsequent clinical trial, the same group of researchers showed an increase in circulating EPCs in SSc patients after treatment with atorvastatin, suggesting a beneficial effect of hydroxymethylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors on vasculogenesis in patients with SSc (20). Even with treatment, however, the number of EPCs did not reach the levels in healthy individuals. Furthermore, the capacity of EPCs to differentiate into mature ECs could not be restored by the application of atorvastatin.

In their study of a larger cohort of SSc patients, del Papa et al (19) identified EPCs via the same cell surface markers as Kuwana et al (22): CD34\(^+\), CD133\(^+\), and VEGFR-2\(^+\) (19). In contrast to the findings of the Kuwana study, the numbers of EPCs in the blood of SSc patients in the del Papa study were found to be significantly increased. Further subgroup analysis revealed a negative correlation between EPCs count and disease duration. Based on this finding, the authors suggested that differences in disease duration might account for the discrepancy between their results and the findings reported by Kuwana et al. Apart from disease duration, no correlations between EPC counts and clinical parameters, including digital ulcers, were observed.

In addition to the numbers of EPCs in the circulation, del Papa et al (19) counted EPCs in the bone marrow of SSc patients and healthy individuals. The number of CD133\(^+\) cells was significantly decreased in the bone marrow of patients with SSc. The ability of bone marrow–derived CD133\(^+\) cells to differentiate into ECs in vitro was found to be reduced in SSc patients. Finally, the number and the size of colonies were reduced, and cells from SSc patients showed morphologic signs of cellular senescence. Of note, the same research group (18) has recently reported that the HMG-CoA reductase inhibitor simvastatin increased the levels of EPCs exclusively in SSc patients with hypercholesterolemia, but not in SSc patients with normal serum cholesterol levels.

Allanore et al (16) assessed EPC counts in the whole blood of patients with SSc, osteoarthritis (OA), and RA, and analyzed potential correlations with clinical parameters. In contrast to previous studies, the authors measured the numbers of CD34\(^+\) and CD133\(^+\) cells but did not analyze the expression of VEGFR-2. The numbers of CD34\(^+\) and CD133\(^+\) cells were increased in patients with SSc as compared with patients with OA. However, the EPC counts in SSc patients were lower than those in RA patients. In patients with SSc, the CD34\(^+\)CD133\(^+\) counts increased in parallel with the European League Against Rheumatism Scleroderma Trial and Research (EUSTAR) group disease activity score. In another study, this group of investigators (17) used VEGFR-2 and lineage (Lin) markers as additional markers to identify EPCs. Furthermore, dead cells could be excluded with the use of the viability marker 7-aminoactinomycin D (7-AAD). EPCs were thus defined as Lin–7-AAD–CD34\(^+\)CD133\(^+\)VEGFR-2\(^+\) cells.

Again, patients with SSc displayed higher numbers of circulating EPCs than did healthy subjects (17). Lower EPC counts in SSc patients were associated with higher Medsger severity scores for SSc and with digital ulcers.

Most recently, Zhu and coworkers (23) found decreased EPC counts in different subsets of SSc patients. Patients with limited cutaneous, diffuse cutaneous, recent-onset, and late-stage SSc all had reduced numbers of EPCs as compared with healthy individuals. EPC counts and EPC function were analyzed by fluorescence-activated cell sorter (FACS) analysis of staining for CD34\(^+\)CD133\(^+\)VEGFR-2\(^+\) cells and colony-forming unit assays, respectively (23). In addition, the authors proposed a new mechanism that might explain the reduced EPC counts in their study. They observed an increased rate of apoptosis in freshly isolated EPCs from patients with SSc. Addition of sera from SSc patients to cultured EPCs from healthy volunteers mimicked these findings and substantially induced apoptosis of EPCs. The proapoptotic effects of SSc sera were abolished by depletion of the IgG fraction, suggesting the presence of anti-EPC autoantibodies in the SSc patient sera. Further experiments revealed that the addition of SSc sera inhibited the phosphorylation of
Akt, which prevented the degradation of FOXO3a (FKHRL-1). Accumulation of FOXO3a, in turn, up-regulated the expression of the proapoptotic protein Bim. Knockdown of FOXO3a and Bim via siRNA strongly reduced the proapoptotic effects of SSc serum (23).

Whether EPC counts are altered in the peripheral blood of SSc patients is still a matter of controversy. The differences in EPC counts between the different studies might be explained by the following reasons: use of different combinations of surface markers, resulting in the analysis of different subsets of EPCs; different methods of handling the dead EPCs; differences in the prevalence of cardiovascular risk factors and in the use of medications by the study patients; or differences in the mean disease duration and severity, which is supported by the inverse correlation of EPC numbers with disease duration and disease activity in some studies (17,19). In contrast to the number of EPCs in SSc patients, functional defects of EPCs in the peripheral blood as well as in the bone marrow have consistently been reported (19,21,22) and indicate a critical role of EPCs in the pathogenesis of SSc, even though the numbers of circulating EPCs might vary (Table 1).

Drugs that increase the number of EPCs and that may at least in part restore vasculogenesis, might offer new therapeutic options for the treatment of vascular disease in SSc. In this context, patients with SSc might potentially benefit from the treatment with HMG-CoA-reductase inhibitors. Injection of in vitro-amplified, autologous EPCs might be another treatment option for patients with severe vascular disease. With regard to the functional defects of EPCs in patients with SSc, the efficacy of these approaches might, however, be limited in SSc. Further identification of the molecular mechanisms underlying these defects is needed in order to develop specific treatment options and restore functional vasculogenesis in patients with SSc.

### EPCs in patients with RA

A growing body of evidence suggests a critical role of impaired vasculogenesis in the pathogenesis of RA, including both reduced numbers and altered functions of EPCs (Table 1). Grisar and coworkers (26) demonstrated a reduction in the numbers of circulating EPCs in patients with RA as compared with healthy controls. As shown by FACS analysis and by colony-forming unit assays, the number of CD133+, CD34+, and VEGFR-2+ EPCs was significantly reduced in RA patients with active disease, as reflected by a Disease Activity Score 28-joint assessment (DAS28) value of ≥3.2. Up-regulation of erythropoietin and VEGF were insufficient to increase the numbers of circulating EPCs in the RA patients. However, no significant differences in EPC numbers and functions between RA patients with low disease activity (DAS28 <3.2) and healthy controls was observed. Cardiovascular risk factors influencing the number of EPCs were equally distributed over the 3 study groups (RA patients with high disease activity, RA patients with low disease activity, and healthy controls). Of note, the numbers of circulating

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**Table 1. Overview of the different roles of EPCs in the pathogenesis of rheumatic diseases**

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<td>Vasculitis</td>
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* EPCs = endothelial progenitor cells; SSc = systemic sclerosis; ECs = endothelial cells; RA = rheumatoid arthritis; DAS28 = Disease Activity Score, 28-joint assessment; VEGF = vascular endothelial growth factor; TNFα = tumor necrosis factor α; SLE = systemic lupus erythematosus; HGF = hepatocyte growth factor; IFNα = interferon-α.
CD133+, CD34+, and VEGFR-2+ EPCs were inversely correlated with the disease activity, as assessed by the DAS28, particularly with the number of swollen and tender joints, but not with the erythrocyte sedimentation rate and the patient's global assessment (26).

Conventional disease-modifying antirheumatic drugs and low doses of glucocorticoids had no effect on the levels of EPCs. However, higher doses of glucocorticoids increased the numbers of circulating EPCs in vivo. Consistent with the increased EPC counts in vivo, the numbers of colonies in the colony-forming unit assay were elevated (25). Treatment with TNFα antagonist also increased the number of circulating EPCs and had beneficial effects on the differentiation and adhesion of EPCs (24,26). Grisar and coworkers (26) reported that EPC counts in patients with RA receiving TNFα inhibitors were similar to the EPC levels in healthy controls, even when the disease was still active. Finally, the number of EPCs was lower in patients with high levels of TNFα as compared with those with low serum concentrations of TNFα, which is consistent with the beneficial effects of TNFα inhibitors.

Silverman et al (30) reported a pronounced recruitment of putative EPCs to the inflamed synovium in several animal models of RA (30). In the collagen-induced arthritis model, numerous cells expressing CD117, a stem cell marker that is found on EPCs, accumulated in inflamed synovial tissues. In addition, exogenously administered EPCs accumulated preferentially in inflamed joints, but not in control joints, when applied in a model of antibody-induced arthritis. Similar results were obtained with human EPCs in the chimeric SCID mouse/human synovial tissue model. The numbers of CD133+ and CD34+ cells were increased by ~3-fold in RA synovial tissue as compared with normal synovial tissue. Silverman and coworkers (30) also observed that blocking antibodies against vascular cell adhesion molecule 1 and α4 integrin potently inhibited the binding of EPCs to activated synovial fibroblasts, suggesting a crucial role of these 2 receptor molecules in the adhesion process of EPCs (30).

In addition to reduced EPC counts, EPC function also seems to be altered in patients with RA (27). Migration of EPCs toward VEGF in a modified Boyden chamber assay was found to be reduced. Furthermore, the adhesion of EPCs to TNFα-treated ECs was impaired as compared with EPCs from healthy donors. However, adhesion to resting ECs and to the extracellular matrix proteins type IV collagen, fibronectin, and laminin was not affected (29). Furthermore, the generation of endothelial cells from CD34+ bone marrow cells after in vitro stimulation with Kit ligand and GM-CSF cells was significantly increased in RA patients as compared with OA patients and with healthy controls (28). Interestingly, the generation of ECs from CD34+ bone marrow cells correlated with the vessel density in the synovium.

Together, these results suggest that many EPCs might become trapped in the synovial tissue in RA, contributing to the increased formation of new vessels in the inflamed joint (82). This might result in a decreased number of circulating EPCs. The bone marrow might not be able to compensate for the emigration of EPCs into the synovial tissue. TNFα seems to be a key player in the impaired vasculogenesis in patients with RA, since it attracts EPCs to the inflamed synovial tissue and induces apoptosis in circulating EPCs. However, the functional defects of EPCs with decreased adhesion and reduced migration of EPCs are not explained by this hypothesis, and therefore have to be investigated in further studies. Although low EPC counts have not yet been established as independent risk factors for cardiovascular disease in RA, the reduced numbers, along with the functional defects, of circulating EPCs might contribute to the increased morbidity and mortality from cardiovascular causes in patients with RA.

Defective vasculogenesis in SLE

SLE is associated with premature and accelerated cardiovascular disease that is not explained by traditional risk factors, such as hypertension, hyperlipidemia, or hyperglycemia. Several groups of investigators have independently reported finding reduced numbers of EPCs in the peripheral blood of patients with SLE (Table 1) (31–34). Although triple staining for VEGFR-2, CD34, and CD133 was not performed in any of these studies, double staining for VEGFR-2 and CD133 or for CD34 and CD133 consistently showed a reduction in the numbers of putative EPCs in patients with SLE, even during inactive stages of the disease.

Similar to the situation in patients with SSc and RA, EPCs may be altered in SLE patients, as demonstrated in colony-forming assays (31,33). EPCs from patients with SLE were also shown to have a reduced capacity to differentiate into mature ECs in vitro. Uptake of low-density lipoprotein, binding of agglutinin I, and expression of von Willebrand factor were found to be reduced. Furthermore, EPCs from patients with SLE released less VEGF and hepatocyte growth factor compared with EPCs from healthy donors (31). EPCs from SLE patients might also be more susceptible to apopto-
sis, which is indicated by increased levels of caspase 9 protein (33).

In SLE, interferon-α (IFNα) may play a central role in perturbed vasculogenesis, since EPC counts correlated inversely with serum levels of IFNα. In this context, the expression of the interferon-inducible gene Mx-1 in peripheral blood mononuclear cells was also inversely correlated with EPC counts (32). IFNα induced apoptosis in EPCs and inhibited differentiation into mature ECs. Of note, the EPCs isolated from the peripheral blood of SSc patients produced increased amounts of IFNα in vitro (31). Inhibition of IFNα or type I IFN receptor by blocking antibodies prevented the functional defects and restored a normal EPC phenotype in vitro. Similarly, inhibition of Toll-like receptor 7 (TLR-7) and TLR-9, which are known to regulate the induction of IFNα, had beneficial effects (31). In summary, defective vasculogenesis with reduced numbers and altered functions of EPCs may contribute to the increased cardiovascular risk observed in patients with SLE. Inhibition of IFNα, TLR-7, and TLR-9 might restore vascular remodeling by EPCs and therefore reduce the cardiovascular risk in patients with SLE.

EPCs for the repair of damaged vessels in vasculitis

De Groot and coworkers (35) examined whether EPCs play a role in the repair of damaged endothelium in antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis (35). In patients with active, untreated disease, the number of EPCs after 4 days in culture did not differ from that in healthy volunteers, as assessed by uptake of low-density lipoprotein and the binding of agglutinin I. No correlations with leukocyte counts, platelet counts, or kidney function were observed. With the induction of disease remission, the EPC counts increased significantly by almost 2-fold. In contrast, circulating ECs were found to be increased during active disease and decreased upon induction of remission. Thus, EPCs might represent markers of vascular regeneration, whereas circulating ECs might reflect disease activity, in the vasculitides.

Summary and conclusions

Vasculogenesis is not restricted to embryonic development, but contributes to the vascular homeostasis and integrity in adults. EPCs induce neovascularization of ischemic tissues and stimulate the repair of damaged vessels by the release of angiogenic factors, integration into the vessel wall, and differentiation into mature ECs. In fact, several cardiovascular risk factors are associated with a reduced number of circulating EPCs, and EPC counts are inversely correlated with the risk of major cardiovascular events, such as myocardial infarction and stroke. Thus far, EPCs have been implicated in the vascular pathogenesis of several rheumatic diseases. Reduced levels of circulating EPCs have been found in patients with RA and SLE. In SSc, however, EPC counts are still unresolved. In addition, altered EPC functions with increased apoptosis, decreased production of angiogenic factors, reduced adhesion, and impaired differentiation capacity have been described in patients with RA, SLE, and SSc. Together, these findings suggest that defects in vasculogenesis might contribute to the increased cardiovascular risk in patients with RA and SLE and might play a critical role in the vasculopathy observed in patients with SSc.

However, a major problem in EPC research is the lack of clear definitions and common protocols for the isolation and identification of EPCs (83). Because of this, comparison of the results of different studies is often difficult, since different EPC isolation procedures might yield different subpopulations and maturation steps of EPCs. The lack of clear definitions and protocols might also explain the differences in EPC counts observed in SSc. Guidelines for the isolation, identification, quantification, and culturing of EPCs, such as those compiled by the EUSTAR group (83), will help to unify research within the field and allow better comparison between studies. Large amounts of blood are needed for the isolation of sufficient numbers of EPCs, and the protocols for the culture and propagation of EPCs are complicated. Consequently, evidence of altered EPC functions in patients with rheumatic diseases is still limited.

Despite great progress in EPC research, many questions remain open. Do different subpopulations of EPCs have distinct functions during vasculogenesis? What is the physiologic relevance of mesenchymal stem cells and tissue-resident stem cells that can differentiate into EPCs? Although key molecules have been identified, the molecular mechanisms that lead to the formation of new vessels and the repair of preexisting vessels are only incompletely understood.

In the field of rheumatology, the levels and function of EPCs have been investigated in SSc, RA, SLE, and the vasculitides. However, a number of research questions are still pending: What are the reasons for the observed functional defects of EPCs? Do patients benefit from injections of autologous EPCs or from treatment with drugs that increase the number of

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circulating EPCs (e.g., HMG-CoA reductase inhibitors) despite functional defects? Are there any long-term side effects of therapeutic injection of EPCs, such as enhanced plaque formation or an increased risk of cancer due to uncontrolled neoangiogenesis? These issues need to be addressed in further studies and might help to improve our understanding about the vascular pathogenesis of rheumatic diseases.

REFERENCES


