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Novel Mediators and Mechanisms in Angiogenesis and Vasculogenesis
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Influence of Cardiovascular Risk Factors on Endothelial Progenitor Cells

Limitations for Therapy?

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***Abstract*—**The ideal way to prevent and cure atherosclerosis and the subsequent end organ damage is to restore and rejuvenate the dysfunctional vasculature and the damaged organs. Various studies have underlined the important role of bone marrow–derived endothelial progenitor cells (EPCs) in vasculogenesis and angiogenesis of ischemic tissue, but only a few studies have concentrated on the role of EPCs in the prevention and therapy of atherosclerosis. Extended endothelial cell damage by cardiovascular risk factors can result in endothelial cell apoptosis with loss of the integrity of the endothelium. The consequences are an increased vascular permeability of the endothelium followed by facilitated migration of monocytes and vascular smooth muscle cell proliferation, resulting in the premature manifestation of an atherosclerotic lesion. A growing body of evidence suggests that circulating EPCs play an important role in endothelial cell regeneration. Systemic transfusion or intrinsic mobilization of EPCs enhances the restoration of the endothelium after focal endothelial denudation, resulting in a diminished neointima formation. In mice with atherosclerotic lesions, bone-marrow–derived stem cells are able to reduce atherosclerotic plaque size. However, various studies have demonstrated that in humans, cardiovascular risk factors impair number and function of EPCs, potentially restricting the therapeutic potential of progenitor cells. The current review focuses on the role of cardiovascular risk factors on endothelial cell apoptosis and EPCs with its pathophysiological consequences for atherogenesis and a regenerative therapy approach and will highlight the role of EPCs as a marker for cardiovascular mortality and morbidity. **(*Arterioscler Thromb Vasc Biol*. 2006;26:257-266.)**

Key Words: endothelial progenitor cells ■ risk factors ■ apoptosis ■ endothelium

Atherosclerosis is the leading cause of death in the Western world. Clinical manifestations of atherosclerosis include myocardial infarction, heart failure, stroke, and peripheral artery disease, resulting in irreversible organ damage. Current guidelines for the prevention of atherosclerotic disease focus on lifestyle modifications and risk factor reduction and to minimize devastating factors such as free oxygen radicals and the subsequent endothelial cell (EC) damage. The recently published INTERHEART study has demonstrated that 9 easily measurable cardiovascular risk factors are associated with >90% of the risk of an acute myocardial infarction in a large global case-control study.[1] Accumulation of risk factors such as smoking, hypertension, and diabetes increased the odds ratio for acute myocardial infarction to 13.01 (99% CI, 10.69 to 15.83) compared with patients without these risk factors. Although the correlation between risk factors and atherosclerosis and resulting myocardial infarction is well known, compliance with lifestyle

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modifications and risk factor reduction is poor. Therefore, novel (regenerative) treatment options are warranted to reduce the incidence of cardiovascular disease.

The current review focuses on the role of cardiovascular risk factors on EC apoptosis and on the regenerative capacity of the organism and highlights potential limitations of a regenerative therapy approach.

Endothelial Progenitor Cells

In 1997, Asahara et al isolated a circulating angioblast from human peripheral blood of adults, which had the potential to differentiate in vitro into ECs and to contribute to neoangiogenesis after tissue ischemia in vivo.[2,3] The so-called endothelial progenitor cell (EPC) is characterized by the surface markers CD34 and vascular endothelial growth factor (VEGF) receptor 2 or kinase domain receptor (KDR). An immature subset of EPCs expresses the surface marker CD133.[4–6] The ability of peripheral blood-derived EPCs to form "late-outgrowth colony-forming units–ECs" underlines the stem cell–like properties and gives information about the clonogenic potential of these cells. The origin as well as the phenotypic and functional characterization of EPCs remain unsettled. Rafii et al distinguish between bone marrow (BM)–residing EPCs and circulating EPCs.[6] In addition, it has been demonstrated that myelomonocytic cells[7] as well as spleen-derived mononuclear cells (MNCs)[8] and cord blood–derived MNCs contribute to the pool of EPCs.[9,10] Various surface markers are expressed on EPCs and are used for EPC characterization.[6] This apparent heterogeneity in cells may reflect different developmental stages of EPCs during the maturational process from the BM residual cell to the mature vascular wall cell. Currently, it is accepted standard to measure the circulating numbers of EPCs by flow cytometry using either antibodies against CD34 and KDR or CD133, whereas the functional, clonogenic capacity should be evaluated using colony-forming unit assays.[6,11]

Recent attempts in cardiovascular research have focused on the regeneration of ischemic and damaged myocardial tissue using various types of stem and progenitor cells.[12–14] Although the regeneration of cardiomyocytes by BM-derived cells is still under debate,[15,16] there is evolving evidence that BM-derived EPCs contribute to the pool of ECs in neoangiogenesis.[2,17,18] Meanwhile, various studies have demonstrated the important role of EPCs in vasculogenesis and angiogenesis of ischemic tissue in peripheral artery disease as well as after myocardial infarction,[2,17–20] but only a few studies have concentrated on the role of EPCs in the prevention and therapy of atherosclerosis.[21–23] This is astonishing because atherosclerosis is the preceding disease inevitably leading to cardiovascular complications such as myocardial infarction and stroke.

Atherogenesis: The Pivotal Influence of Risk Factors on the Endothelium

Despite intense research efforts, the underlying molecular mechanisms of atherosclerosis are still incompletely understood. According to the response-to-injury hypothesis, cardiovascular risk factors induce a chemical or mechanical injury of the endothelium that triggers and enables the concomitant invasion of macrophages and lipid deposition.[24] The continuous damage of the vascular endothelium finally results in endothelial dysfunction.[25] The latter is a prerequisite of atherosclerosis and influences the outcome of patients at cardiovascular risk.[26–28] On the molecular and cellular level, endothelial dysfunction is characterized by reduced NO bioavailability[29] and by a progressive loss of ECs.[30] Therefore, damage of the endothelium by inflammation or mechanical or biochemical damage may represent an early, causative event, compromising EC capabilities regulating vascular function and homeostasis. From experimental models, we know that vascular smooth muscle cell (VSMC) proliferation, a crucial step in atherogenesis, is regulated by the endothelium. Denudation of the endothelial monolayer is associated with increased proliferation of VSMCs, leading to neointima formation.[31] The enhancement of re-endothelialization can prevent this detrimental proliferation of smooth muscle cells. In humans, extended EC damage by cardiovascular risk factors can result in EC apoptosis with loss of integrity of the

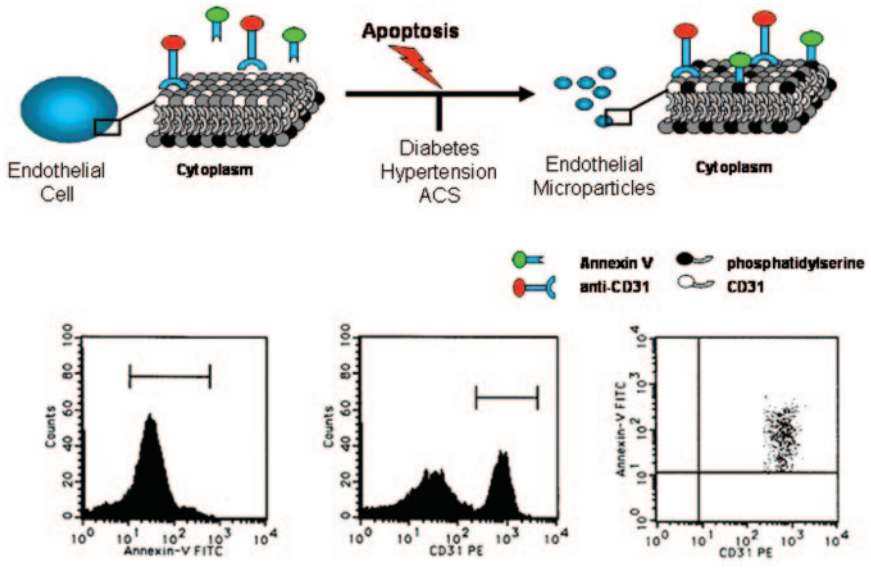

Figure 1. Endothelial cell apoptosis is associated with the release of small membrane particles, the so-called endothelial microparticles. During cell apoptosis, the negatively-charged phosphatidylserine normally located in the inner cytoplasmic membrane becomes surface-exposed at the outer membrane. Fluorescent-labelled annexin V can then bind to the negatively-charged phosphatidylserine. Circulating endothelial microparticles can be quantified in vivo by flow cytometry using annexin V and endothelial surface markers of the mother cell (eg, CD31, CD51, CD62E, CD146, and other endothelial cell–related surface marker).

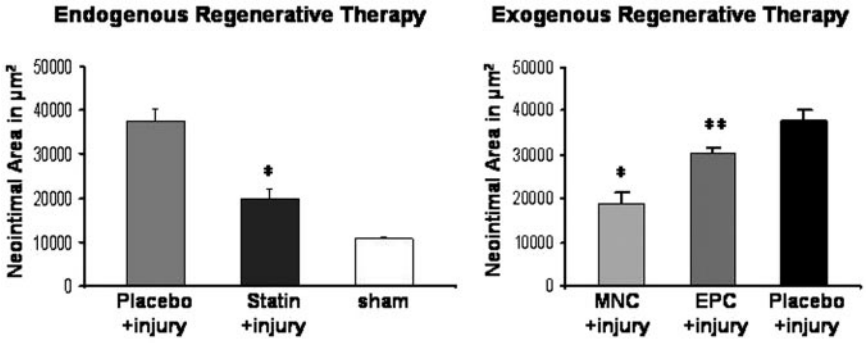

Figure 2. Endothelial progenitor cells accelerate reendothelialization after endothelial cell denudation with concomitant reduction of neointima formation. Neointima area of injured mouse carotid arteries is significantly reduced after statin-induced mobilization of bone marrow–derived endothelial progenitor cells (endogenous therapy, left) and intravenous transfusion of spleen-derived endothelial progenitor cells and total mononuclear cells (exogenous therapy, right). Values are mean±SEM. *$P<0.05$, **$P<0.0001$. n=5 to 8 mice.

endothelium. The consequences equal the sequelae in the experimental model: increased vascular permeability of the endothelium is followed by VSMC proliferation, facilitated migration of monocytes with lipid deposition, and activation of proinflammatory cytokines, resulting finally in the irreversible manifestation of an atherosclerotic lesion.

EC Apoptosis: Integrative Marker of EC Damage?

If EC damage is crucial as the initial step in atherogenesis, quantification of EC death in patients will be helpful as an integrative marker of the detrimental effects on the endothelium. EC apoptosis is associated with conformational changes of the plasma membrane, condensation of the nucleus, followed by DNA fragmentation and the release of small membrane particles, the so-called endothelial microparticles.[32] During cell activation or apoptosis, the negatively charged phophatidylserine normally located in the inner, cytoplasmic membrane becomes surface exposed at the outer membrane (Figure 1). Microparticles bear various antigens derived from their mother cell. EC-derived microparticles (EMPs) have been shown to express CD31, CD51, CD62E, CD146, and other EC-related surface markers.[32] Circulating EMPs can be quantified in vivo by flow cytometry[33] using the negatively charged phosphatidylserine, which binds to fluorescent-labeled annexin V (Figure 1). Elevated EMP levels have been described in conditions of severe EC damage, including thrombotic thrombocytopenic purpura, diabetes,[34] arterial hypertension,[35] acute coronary syndromes,[36] and after myocardial infarction.[37] In patients with coronary artery disease (CAD), the number of circulating EMPs positively correlate with the severity of coronary endothelial dysfunction, suggesting that endothelial-dependent vasodilatation relies closely on the degree of EC apoptosis.[38] The importance of microparticles in cardiovascular disease is further supported by their functional properties. Functional characteristics of microparticles include their procoagulant activity, their involvement in inflammation and their direct effect on endothelial function.[39]

EC Regeneration by EPCs

Under physiological conditions, EC apoptosis presumably leads to increased EC turnover, resulting in the repair of the damaged endothelium. Until recently, EC repair mechanisms were thought to be exclusively mediated by the adjacent EC. However, adult blood vessels regenerate only moderately in adults under physiological conditions. The half life of an adult EC has been reported to be 3.1 years.[40] A growing body of evidence suggests that circulating EPCs play an important role in EC regeneration.[3,41] We and others have demonstrated that systemic transfusion or intrinsic mobilization of EPCs enhances the restoration of the endothelium after focal endothelial denudation, resulting in a diminished neointima formation[8,22,23] (Figure 2). Furthermore, in a model of disseminated, hyperlipidemia-induced EC damage, systemic transfusion of EPCs improves endothelial dysfunction, indicating an important role of EPCs in the reconstitution of damaged endothelium (unpublished data, 2004). Finally, in mice with atherosclerotic lesions, BM-derived stem cells are able to reduce atherosclerotic plaque size.[21] However, a recent report demonstrated that stem and progenitor cell treatment in mice may result in increased plaque size and may have detrimental effects on plaque stability.[42] These findings may be explained by increased plaque angiogenesis or the contribution of smooth muscle cell progenitors, which have been shown to increase lesion size.[43] However, human studies clearly demonstrate that high EPC levels are associated with reduced cardiovascular event rates underlining the vasculoprotective action of EPCs.[44,45]

The rejuvenation of the endothelium by circulating EPCs may represent a novel approach in the prevention of atherosclerotic disease. However, limitations in therapy may come from the negative influence of cardiovascular risk factors, which are apparently overwhelming the organism's repair mechanisms, bringing the equilibrium between regeneration and apoptosis out of balance.

EPCs and Cardiovascular Risk Factors

Small clinical studies have shown that the number of circulating EPCs inversely correlates with risk factors for atherosclerosis.[11,46] Circulating CD34/KDR-positive progenitor cells are reduced to ≈50% in patients with CAD compared with control groups. In addition, EPCs isolated from patients with CAD displayed an impaired migratory response, which was inversely correlated with the number of cardiovascular risk factors.[46]

Arterial Hypertension

In patients with arterial hypertension, systolic blood pressure negatively correlates with the number of circulating CD133+ and CD34+/KDR+ EPCs, whereas the clonogenic potential (number of colony forming units–ECs) is not impaired by

arterial hypertension (Werner, unpublished data, 2004).[46]) Experimental data demonstrate that angiotensin II, a potent mediator of detrimental effects in arterial hypertension, can accelerate the onset of EPC senescence by gp91 phox-mediated increase in oxidative stress, leading to an impaired proliferation activity of EPCs. Angiotensin II–induced EPC senescence was inhibited by treatment with the angiotensin II type 1 receptor blocker valsartan.[47] In patients with CAD, 5 mg ramipril per day resulted in a sustained increase in circulating EPCs (maximum 2.5-fold).[48] Ramipril was able to improve proliferation, migration, and in vitro vasculogenesis in this patient cohort. These results were confirmed in the Endothelial Progenitor Cells in Coronary Artery Disease (EPCAD) study, demonstrating that angiotensin-converting enzyme (ACE) inhibitor treatment was associated with increased numbers and improved clonogenic potential of circulating EPCs compared with patients not on ACE inhibition (Werner, unpublished data, 2004).

Diabetes

Recent studies have underlined the detrimental effects of types 1 and 2 diabetes on EPC function.[49,50] Tepper et al demonstrated that in type 2 diabetes proliferation capacity of EPCs was reduced, adhesion capacity on activated human ECs was impaired, and diabetic EPCs showed reduced tube formation in a Matrigel assay. Interestingly, hemoglobin A1C negatively correlated with EPC proliferation and in vitro EPC number in types 1 and 2 diabetes. In this context, hyperglycemia was identified to mediate the detrimental effects on EPCs by a decrease in NO production and matrix metalloproteinase-9 activity.[51] In general, diabetes seems to impair the functional properties and the mobilization of BM-MNCs. Mobilization of BM cells to the peripheral blood is significantly impaired in an experimental model of diabetes and results in an abrogated revascularization after hindlimb ischemia.[52] However, placenta growth factor, a potent proangiogenic agent, was able to increase EPC differentiation from diabetic BM-MNCs by 6-fold, antagonizing the detrimental effects of diabetes. In a prospective, double-blind study in 18 patients with type 2 diabetes, 40 mg olmesartan increased circulating EPC counts but did not affect hematopoietic progenitor cells.[53] This underlines a pivotal role of the renin-angiotensin system in EPC mobilization, which can at least modify the detrimental effects seen on EPC number and function in diabetes.

Hyperlipidemia

One of the most important cardiovascular risk factors is the increased low-density lipoprotein (LDL) cholesterol concentration. However, only few studies have investigated the influence of LDL cholesterol[54–57] and none that of high-density lipoprotein cholesterol (HDL-C) on EPC number and function. Hypercholesterolemia was associated with reduced EPC numbers in 20 age-matched patients with hypercholesterolemia.[54] Proliferative capacity, migratory activity, and in vitro vasculogenesis were negatively influenced by hypercholesterolemia. The underlying mechanisms are probably an increased rate of EPC senescence/apoptosis, as demonstrated after incubation of EPCs with oxidized LDL.[56] These effects were prevented by incubation of EPCs with 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors (statins). The underlying molecular mechanisms of the protective effect of statins on EPC number and function were identified by Dimmeler et al in 2001, who demonstrated a phosphatidylinositol 3-kinase/Akt-dependent pathway responsible for the increase in EPC numbers after statin treatment.[58] To elucidate the role of HDL-C, we could demonstrate that increased HDL-C directly correlated with EPC numbers in patients with CAD, indicating that at least part of the vasculoprotective action of HDL-C may be mediated by EPCs (Werner, unpublished data, 2004).

Smoking

Smoking is known to have detrimental effects on the cardiovascular system. Interestingly, nicotine has been associated with increased neovascularization.[59] Smoking has been identified as an important risk factor for reduced EPC numbers in one of the first studies on cardiovascular risk factors by Vasa et al.[46] However, Wang et al recently demonstrated that the role of nicotine is more complex than initially expected.[60] In an experimental study, they demonstrated that low concentrations of nicotine ($10^{-8}-10^{-12}$ mol/L) increased EPC number and activity, whereas higher (toxic) concentrations ($>10^{-6}$ mol/L) were associated with cytotoxicity. In humans, Kondo et al demonstrated that chronic smokers (n=15) exhibit reduced EPC levels that can be restored after smoking cessation within 4 weeks.[61] There was no difference between patients who received a nicotine patch for smoking cessation compared with patients without patch, questioning the direct effects of nicotine on EPC counts at least in vivo.

Physical Inactivity

Regular physical activity has been identified as an important predictor for reduced cardiovascular mortality and morbidity. In contrast, physical inactivity has been associated with the increased occurrence of various cardiovascular diseases, including CAD, and is associated with increased oxidative stress, endothelial dysfunction, and atherosclerosis in experimental models.[62] We know from experimental data that mice with regular physical activity in a running wheel show significantly higher numbers of circulating EPCs compared with mice subjected to a sedentary lifestyle in a conventional setting. The increase in circulating progenitor cells was associated with an enhanced re-endothelialization after focal EC damage, which resulted in a reduced neointima formation.[63] In humans, a significant increase in progenitor cell numbers was observed in patients who resumed a standardized physical activity during a rehabilitation program,[63] in patients with CAD,[64] and in healthy individuals exercising for ≥30 minutes.[65]

Other Risk Factors

Various other cardiovascular risk factors have been associated with reduced EPC numbers and function. In addition to a family history of premature CAD, this includes homocysteine[66] and C-reactive protein (CRP).[67,68] The latter has been shown to be an important marker of inflammation associated with endothelial dysfunction and atherosclerosis. When cul-

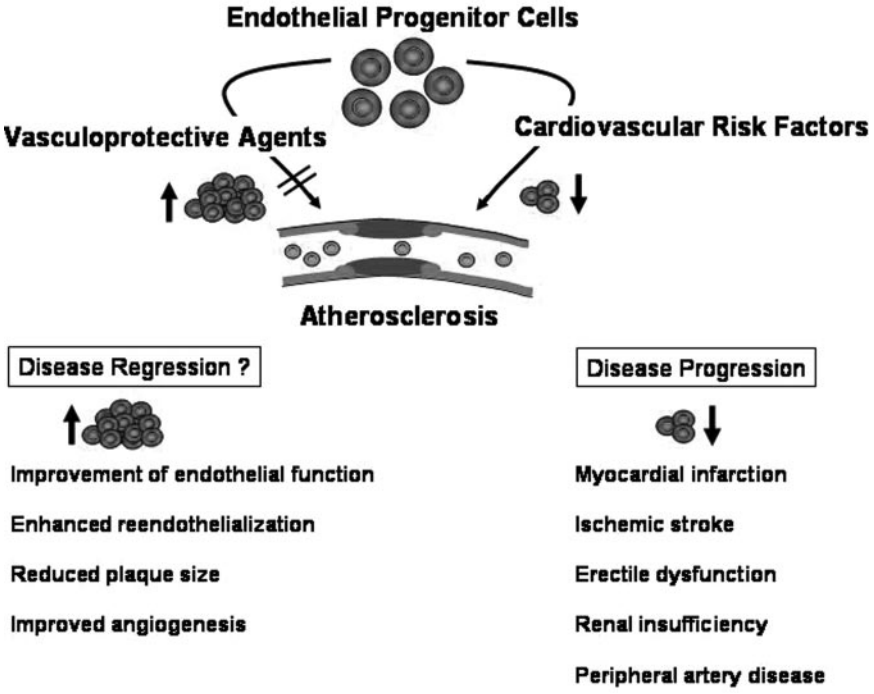

Figure 3. Vasculoprotective agents increase the number and function of endothelial progenitor cells improving endothelial function and preventing progression of atherosclerosis. A risk factor–mediated decrease in number and function of endothelial progenitor cells is associated with atherosclerotic disease.

tured EPCs are incubated with CRP >15 μg/mL, EPC numbers in vitro are significantly reduced compared with controls and endothelial surface markers such as lectin or vascular endothelial (VE)-cadherin vanish.[68] The in vitro angiogenesis potential was significantly impaired after CRP incubation; however, this effect could be antagonized by cotreatment with the peroxisome proliferator-activated receptor-γ agonist rosiglitazone. In addition to the detrimental effect of CRP on the adhesive capacity of EPC, CRP was able to downregulate mRNA expression of monocyte chemoattractant protein-1 (MCP-1), MCP-2, macrophage inflammatory protein-1 (MIP-1), colony stimulating factors, and interferon-inducible protein-10 (IP-10).[68] Suppressors of cytokine signaling (SOCS) 2 and 3, recently identified inhibitors of the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway that regulate cellular growth, differentiation, and hematopoiesis, are highly upregulated in EPCs. The CRP-mediated upregulation of SOCS proteins may inhibit the JAK/STAT pathway, resulting in the functional impairment of the EPC cytokine release, which has been postulated to be an important function of EPCs in arteriogenesis and re-endothelialization.[68]

EPCs and Cardiovascular Disease

In addition to cardiovascular risk factors, several cardiovascular diseases have been associated with impaired number and function of circulating EPCs[20,69–76] (Figure 3). All conditions of manifest atherosclerotic disease are accompanied by reduced EPC numbers and migratory capacity.[46] Most likely, the observed impairment of progenitor cells in these patients is attributable to the accumulation of cardiovascular risk factors resulting in a reduced regenerative potential.[46] Hill et al demonstrated a strong correlation between the number of circulating EPCs and the patient's combined Framingham risk factor score.[11] Levels of circulating EPCs represented a better predictor of endothelial function than conventional risk factors.[11]

Acute coronary syndromes and acute myocardial infarction go along with elevated numbers of EPCs indicating that EPC-mediated tissue and vessel repair is a "physiological" response of the organism after severe ischemia.[77–79] However, according to our own observations, these mobilized EPCs are functionally impaired (Werner, unpublished data, 2004). Similar results have been obtained in patients with congestive heart failure. Heeschen et al demonstrated that the in vivo proangiogenic potential of human BM-MNCs in a mouse model of hindlimb ischemia is significantly impaired if cells are derived from patients with ischemic heart disease.[72] This was mainly triggered by a reduced migratory capacity and impaired clonogenic potential of BM-MNCs.

In patients with stroke EPC counts are significantly reduced compared with control subjects.[75] The level of EPCs correlates with the Framingham coronary risk score, indicating that low EPC numbers may play a role in the pathophysiology of cerebrovascular disease.[75] Furthermore, analysis of patients with cerebral artery occlusion revealed a positive correlation between circulating EPCs and regional blood flow in areas of chronic hypoperfusion.[76]

In studies investigating EPC levels and function in patients with chronic renal failure but no clinical evidence for CAD, renal insufficiency was associated with a marked decrease in circulating EPCs and colonies.[69,70] These findings appeared irrespective of concomitant cardiovascular risk factors. However, renal insufficiency is known to be a risk factor associated with an increased incidence of atherosclerotic disease. Surprisingly, patients with active rheumatoid arthritis have been shown to have a reduced pool of circulating EPCs, which is significantly higher when patients receive tumor necrosis factor blocker therapy.[80] It is tempting to speculate that the chronic inflammation impairs EPC number and

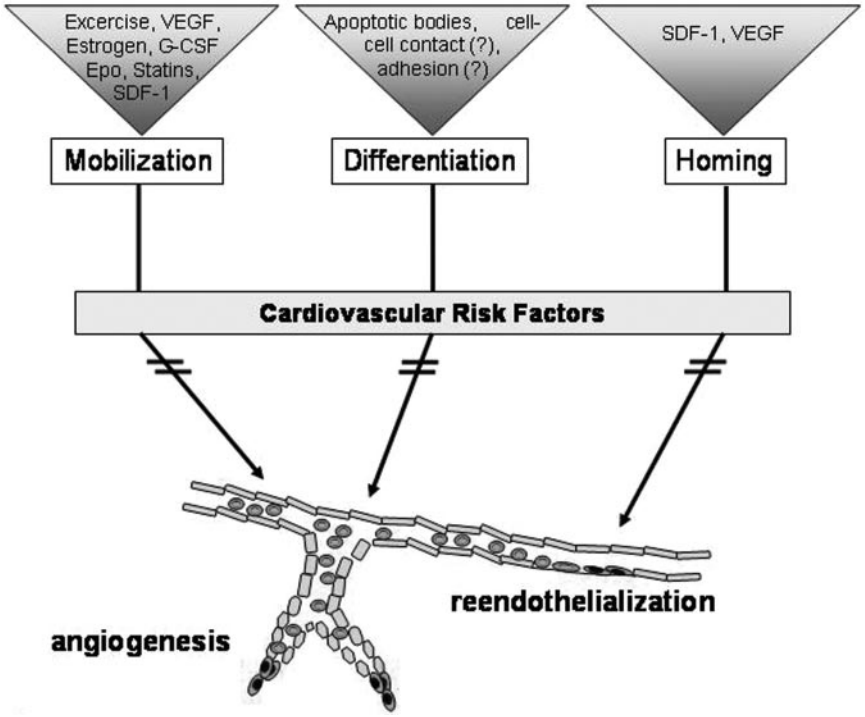

Figure 4. Endothelial progenitor cell–mediated angiogenesis and reendothelialization requires effective mobilization, differentiation, and homing of bone marrow cells. Various agents have been shown to positively influence these steps whereas cardiovascular risk factors may have significant negative impact on mobilization, differentiation, and homing processes.

function, which accounts for the increased cardiovascular mortality and morbidity observed in patients with rheumatoid arthritis. Finally, a small-scale study has demonstrated that in patients with erectile dysfunction, the number of $CD133^+$ progenitor cells is reduced compared with controls, indicating that impaired EPC-mediated regeneration of the endothelial monolayer in endothelial dysfunction may indeed play an important role in the development of atherosclerosis-associated diseases. Furthermore, in patients with established CAD, the number of circulating $CD133^+$ EPCs is an independent predictor for erectile dysfunction underlining the important EPC-mediated link between cardiovascular risk factors and endothelial and erectile dysfunction (Baumhäkel and Werner, unpublished data, 2006).

EPC-Mediated Vasculoprotection

The presented results suggest that cardiovascular risk factors play a pivotal role in influencing the number and function of circulating EPCs. All major known cardiovascular risk factors negatively influence number of EPCs, migratory capacity, as well as the clonogenic potential of progenitor cells. In patients with manifest atherosclerotic disease, one may speculate that the continuous detrimental effects of risk factors on circulating EPCs result in an impairment of the organism's regenerative capacity, with the result of an atherosclerotic disease. This implicates that on the other hand, improvement of number and function of EPCs may result in an effective vasculoprotection preventing the initiation and progression of atherosclerosis. As already mentioned above, there is good evidence that statins and ACE inhibitor mediate at least part of their pleiotrophic, vasculoprotective action via EPCs[23,48,58,74,81] (Figure 4). In addition, physical activity[63–65] and estrogens[82] have been shown to influence EPC number and function. In ovariectomized mice, EPCs are significantly reduced, and re-endothelialization after vascular injury is impaired, resulting in an enhanced neointima formation.[82] Estrogen substitution completely normalizes EPC counts and restores the re-endothelialization capacity. Interestingly, in women with artificially high estrogen concentrations in preparation for in vitro fertilization, EPC numbers are significantly increased compared with a control group.[82] Experimental data demonstrate that systemic transfusion of healthy EPCs in conditions of arterial EC denudation can enhance re-endothelialization, resulting in a diminished neointima formation.[8] Furthermore, transfusion of healthy MNCs or EPCs derived from wild-type mice in apolipoprotein E knockout mice can improve hypercholesterolemia-induced endothelial dysfunction and the development of atherosclerosis (Wassmann, unpublished data, 2006).[21] Interestingly, Dernbach et al and He et al independently demonstrated that EPCs are equipped with antioxidative enzyme systems, allowing an improved survival of cells in conditions of severe oxidative stress. High intrinsic expressions of manganese superoxide dismutase as well as catalase and glutathione peroxidase were identified as a critical mechanism protecting EPCs against oxidative stress. These results suggest that EPCs are equipped with efficient protection systems, making these cells even more attractive for cell therapy.

EPCs and Cardiovascular Mortality and Morbidity

Apparently, cardiovascular risk factors negatively influence EPC number and function, whereas vasculoprotection is at least in part mediated by functionally active EPCs. Therefore, one may speculate that EPCs represent a cellular risk marker, integrating the positive and negative mediators affecting the endothelial monolayer. To evaluate the prognostic value of circulating EPCs, we performed the EPCAD study in which

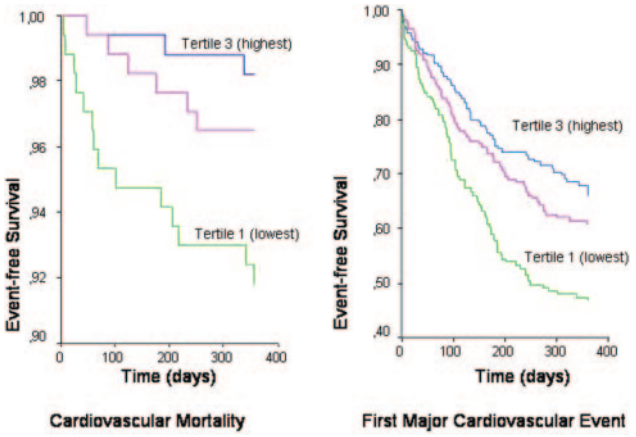

Figure 5. Kaplan–Meier curves showing the incidence of cardiovascular death ($P=0.013$) and the occurrence of a first major cardiovascular event (myocardial infarction, hospitalization, revascularization, and cardiovascular death; $P<0.001$) at 12 months according to the tertiles of circulating $CD34^+/KDR^+$ endothelial progenitor cells at the time of enrollment.

the number of $CD34^+/KDR^+$ EPCs was determined by flow cytometry in 519 patients with angiographically documented CAD.[44] The association between EPC baseline levels and cardiovascular mortality, the occurrence of a first major cardiovascular event (myocardial infarction, hospitalization, revascularization, and cardiovascular death), revascularization, hospitalization, and all-cause mortality after 12 months was evaluated. The cumulative event-free survival increased stepwise across tertiles of baseline EPC levels for cardiovascular mortality, first major cardiovascular event, revascularization, and hospitalization (Figure 5). After adjustment for age, gender, vascular risk factors, drug therapy, percutanous coronary intervention, left ventricular ejection fraction, and concomitant disease, increased EPC levels were associated with a lower risk for cardiovascular death (hazard ratio [HR], 0.31; 95% CI, 0.16 to 0.63; $P=0.001$), first major cardiovascular event (HR, 0.74; 95% CI, 0.62 to 0.89; $P=0.002$), revascularization (HR, 0.77; 95% CI, 0.62 to 0.95; $P=0.017$), and hospitalization (HR, 0.76; 95% CI, 0.63 to 0.94; $P=0.012$). The results of the EPCAD study clearly demonstrate that the level of circulating $CD34^+/KDR^+$ EPCs predicts the occurrence of cardiovascular events and cardiovascular death. Similar results were obtained in a patient population including healthy control subjects, patients with stable CAD, and patients with acute coronary syndromes.[45]

Evaluating EC Apoptosis and Regeneration in Patients

Given these results, one may speculate that enhancing the regenerative capacity of the organism may result in an effective prevention of atherosclerosis. However, the situation at the vascular wall is more complex. Increasing evidence suggests that the balance of EC apoptosis and EC regeneration may determine the degree and progression of atherosclerosis (Figure 6). Hristov et al demonstrated that at least in vitro apoptotic microparticles influence EPC migration, suggesting a close interaction between EPC and EC apoptosis at the vascular wall.[83] The definition of a vascular repair index consisting of markers for EC regeneration and apoptosis may be helpful to mimic the situation at the endothelial monolayer. Furthermore, a vascular repair index may be useful as an exact risk-predicting tool and may be possibly helpful for treatment monitoring.

Therapeutic Chances and Limitations

Various experimental studies and some uncontrolled clinical studies have recently demonstrated that BM-derived or peripheral blood-derived EPCs significantly contribute to neoangiogenesis after tissue ischemia. This has been demonstrated for transfused cells and for endogenously mobilized EPCs. However, given the results shown above, it is likely that the observed (positive) effects after autologous transfusion or mobilization of EPCs in patients with cardiovascular risk factors and cardiovascular disease are limited because of

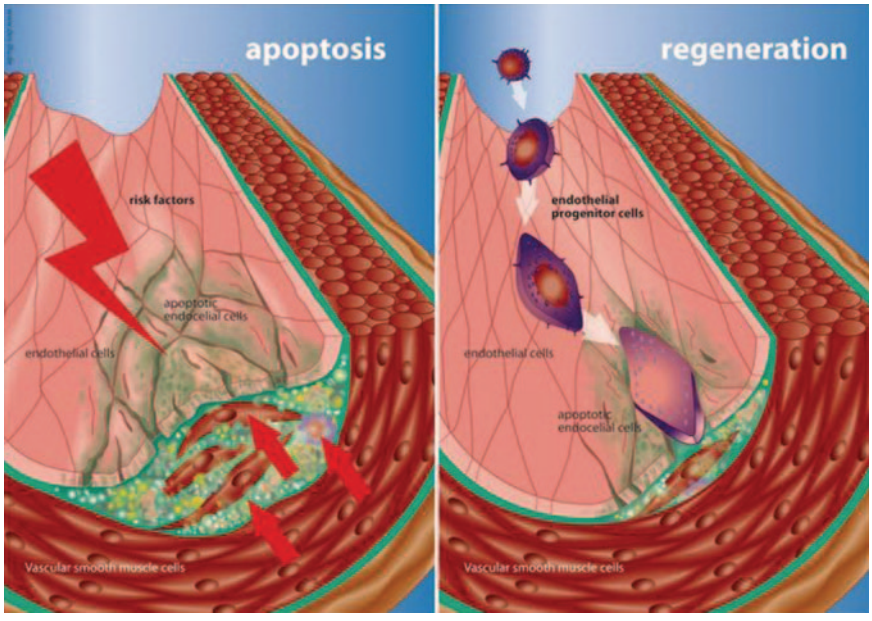

Figure 6. The balance between endothelial cell apoptosis and endothelial cell regeneration may determine the degree and progression of atherosclerosis. The definition of a vascular repair index consisting of markers for endothelial cell regeneration and apoptosis may be helpful to mimic the situation at the endothelial monolayer.

a significant impairment of cells. Because allogeneic transfusion of progenitor cells from healthy donors bears the problem of immunologic incompatibilities, selective modulation of EPC mobilization and cell function appears to be the future strategy. First attempts have been made using erythropoietin, VEGF, G-colony stimulating factor, and stromal-derived growth factor-1[84–90] (Figure 4). For example, erythropoietin treatment has been shown to increase number, proliferation, and migration of mouse embryonic bodies, ECs from embryonic bodies, and human EPCs.[86] However, besides these promising results, we have no data available showing beneficial effects of the described substances in patients with risk factor–impaired progenitor cells. Therefore, future research will have to focus on the identification of the molecular mechanisms associated with risk factor–mediated dysfunction of EPCs.

First hints that EPC therapy may indeed improve survival in atherosclerotic disease come from mathematical models. Kravchenko et al estimated the impact of progenitor cell therapy for atherosclerosis on cardiovascular mortality, life expectancy, and survival compared with the lifetime control of conventional risk factors.[91] In this model, progenitor cell therapy was applied at the age of 30 assuming a 10-year delay in atherosclerosis progression. Males receiving EPC therapy for atherosclerosis had the lowest projected cardiovascular mortality rate compared with patients with an "ideal" lifetime control of risk factors. Simulated progenitor cell therapy showed an effect on life expectancy better than the complete elimination of cancer (in males, an additional 5.94 versus 2.86 years). This simulation study suggests that it may be promising to search for a sufficient way to rejuvenate the endothelium to prevent atherosclerosis. However, the crucial question of how to treat patients remains. Should we keep on trying to isolate progenitor cells from peripheral blood or BM and retransfuse them, or are we in need of studies evaluating methods for intrinsic stem cell mobilization? For the former, we definitely need methods of cell engineering or "simple" lifestyle modifications to improve the functionality of risk factor–damaged, isolated cells. For the latter, we have to admit that the "ideal" substance that mobilizes and activates progenitor cells and by the same time allows selective tissue homing has not been defined yet. Presumably, we are in need for a "cocktail" of cytokines, hormones, and growth factors to achieve the goal of selective tissue regeneration (Figure 4).

Strengthening the regenerative capacity of the organism seems one way to reduce the incidence of atherosclerosis. Alternatively, various studies have underlined the importance of risk factors on EC apoptosis induction. Measurement of circulating endothelial microparticles may serve as a powerful tool, allowing us to mirror the actual detrimental effects of risk factors on the endothelium with one integrative marker. This may have important therapeutic implications. Patients with severe EC damage but favorable regenerative potential may show more benefit from risk factor reduction or antiapoptotic therapies, whereas patients with an impaired regenerative potential but moderate EC apoptosis may be in the need for a regenerative therapy. The therapeutic goal must be the equalization of the imbalance between EC regeneration and apoptosis. In the future, the use of a vascular repair index may be important for choosing therapy strategies with a maximized benefit for the patient. With this knowledge in mind, we need to search for more effective antiapoptotic, proregenerative therapy strategies not only for neoangiogenesis but, more important, for regeneration of the dysfunctional vascular wall, which represents the common trunk for all cardiovascular diseases.

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