Significance of Endothelial Progenitor Cells in Subjects With Diabetes

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Diabetes complications represent a huge burden for patients and health services. The fight against each single complication has led to significant improvements in diabetes care, especially for microvascular complications, yet macroangiopathy remains a major source of morbidity and mortality. A common approach for the prevention and treatment of diabetes complications relies on the understanding of their complex pathophysiology. A unifying biochemical theory suggests that oxidative stress underlies subsequent cellular damage pathways, which leads to diabetes complications, but common supracellular mechanisms are still unclear. Endothelial progenitor cells (EPCs) are circulating immature cells that contribute to vascular homeostasis and compensatory angiogenesis. During the last decade, data have become available indicating that alterations in EPCs may have an important causative role in the development and progression of virtually all diabetes complications. In this review, we will focus on the mechanisms of EPC reduction and dysfunction associated with diabetes by discussing their role in each single complication and possible therapeutic interventions.

A unified pathogenesis of late diabetes complications

Diabetes is associated with a unique constellation of disabling complications. While it was originally thought that a single patient tends to develop the cluster of micro- or macrovascular complications, recent prospective studies show that typical markers of microvascular dysfunction, such as microalbuminuria or retinal vascular abnormalities, are associated with an increased risk of macrovascular events (1,2). These and other data suggest that there must be a unifying pathogenetic model underlying diabetes complications. To date, the most credited and supported model proposes that oxidative stress originating from mitochondria activates all subcellular damage pathways (3). However, subsequent events diverge for each complication, and there is not a supracellular unifying hypothesis.

The discovery that a subset of circulating immature cells contributes to vascular homeostasis has been a major achievement in many fields of basic science. In this review, we will focus the attention on EPCs, emphasizing their impressive role in virtually all diabetes complications.

EPCs

EPCs were discovered in 1997 as circulating cells with the ability to differentiate into mature endothelium and take part in neoangiogenesis (4). EPCs share markers of hematopoietic (CD34 and CD133) and endothelial (KDR, CD31, and vWf) lineages (5), are derived from bone marrow, and can be mobilized to the peripheral circulation in response to many stimuli (6). Tissue ischemia, through the release of growth factors and cytokines, mobilizes EPCs, which, once in the peripheral circulation, specifically home on the ischemic sites to stimulate compensatory angiogenesis (7). Moreover, EPCs constitute a circulating pool of cells able to form a cellular patch at sites of endothelial injury, thus contributing directly to the homeostasis and repair of the endothelial layer (Fig. 1). Taken together, these observations suggest that EPCs have a major role in cardiovascular biology; in fact, the extent of the circulating EPC pool is now considered a mirror of cardiovascular health. Virtually all risk factors for atherosclerosis have been associated with decrease and/or dysfunction of circulating EPCs (8), while an expanded EPC pool is associated with a decreased cardiovascular mortality (9).

The study of EPC biology consists of two related aspects: quantitative evaluation of the EPC pool and functional assessment. Circulating EPCs can be quantified directly ex vivo using flow cytometry, which is considered the gold standard for this purpose (10); typical surface antigens to identify EPCs are CD34, CD133, and KDR. Functional characteristics are explored in vitro using standardized protocols (11). Proliferation refers to the ability of EPCs to expand and form colonies in culture: EPCs should proliferate in response to growth factors released locally after vascular damage or tissue ischemia. Adhesion is a further step required for both reendothelization and angiogenesis; it is assessed as the ability of EPCs to adhere to a monolayer of mature endothelium in culture. Migration of EPCs through the extracellular matrix is crucial for the growth of new vessels and is generally assessed in vitro as the ability to invade the lower side of a Boyden-like chamber. Finally, after EPCs have adhered to the vessel wall, migrated into the interstitium, and expanded locally, they should spatially organize to form vascular structures; this property can be assessed in vitro as a tube formation assay in which EPCs are seeded with human umbilical vein endothelial cells on a gel of extracellular matrix proteins. All these functions are relevant to the comprehensive role of EPCs, and their integrity can be explored as a whole using an in vivo assay in which EPCs are transplanted into a small labo-

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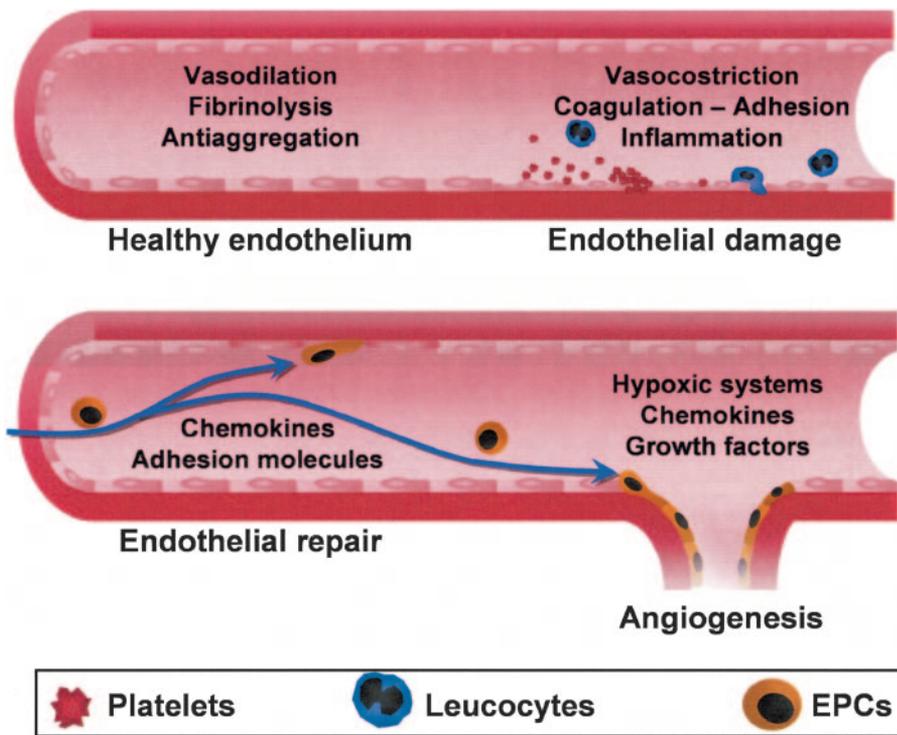
Figure 1—*The contribution of EPCs in the setting of endothelial damage and angiogenesis.*

DNA regions and promotes the transcription of oxygen-sensible genes that encode, among others, vascular endothelial growth factor (VEGF), stromal-derived factor (SDF)-1, and erythropoietin (15). Then, growth factors allow EPCs to undergo transendothelial migration and to pass into the peripheral blood by means of attenuating stromal cell–stem cell interactions and by rearranging extracellular matrix. Once in the bloodstream, progenitor cell recruitment is mediated by hypoxic gradients via HIF-1–induced expression of SDF-1 (16). It has been shown that the expression of angiogenic factors VEGF and HIF-1α are reduced in the hearts of diabetic patients during acute coronary syndromes and that myocardial infarct size in the rat is increased due to a reduced expression of HIF-1α (17,18). Therefore, poor collateral formation in diabetes may be attributed to weaker bone marrow stimulation from the ischemic tissue. We have recently confirmed this hypothesis, showing that bone marrow mobilization of EPCs after ischemia-reperfusion injury is defective in diabetic rats. Inability to mobilize EPCs was associated with downregulation of HIF-1α and weakened release of marrow-stimulating factors, such as VEGF and SDF-1, ultimately leading to insufficient compensatory angiogenesis (19). Another study has shown that progenitor cell mobilization restored blood flow in diabetic mice (20). It is conceivable that HIF-1α

ratory animal subjected to experimental endothelial damage or tissue ischemia. This will definitively demonstrate the global function of EPCs isolated from a given patient or cultured in a specific condition.

EPC alterations in diabetes

Both cytometric and culture methods have extensively demonstrated that type 1 and type 2 diabetic patients have less circulating EPCs than matched healthy subjects. Moreover, diabetic EPCs display functional impairment, such as reduced proliferation, adhesion, migration, and incorporation, into tubular structures (12–14).

The mechanisms underlying EPC reduction in diabetes include weak bone marrow mobilization, decreased proliferation, and shortened survival in peripheral blood (Fig. 2).

The release of EPCs from bone marrow in response to mobilizing stimuli depends on complex interactions in the local marrow microenvironment. Tissue ischemia is considered the strongest stimulus for EPC mobilization, through the activation of hypoxia-sensing systems, such as hypoxia-inducible factor (HIF)-1. HIF-1 is a heterodimeric transcription factor composed of α (HIF-1α) and β (HIF-1β) subunits. While HIF-1β is constitutively expressed, HIF-1α expression

is regulated by cellular oxygen concentrations. Under normoxic conditions, HIF-1α is rapidly degraded via the ubiquitine-proteasome pathway, while cellular hypoxia inhibits its ubiquitination and proteasomal degradation, allowing HIF-1α to dimerize with HIF-1β. The resulting active HIF-1 binds to enhancer
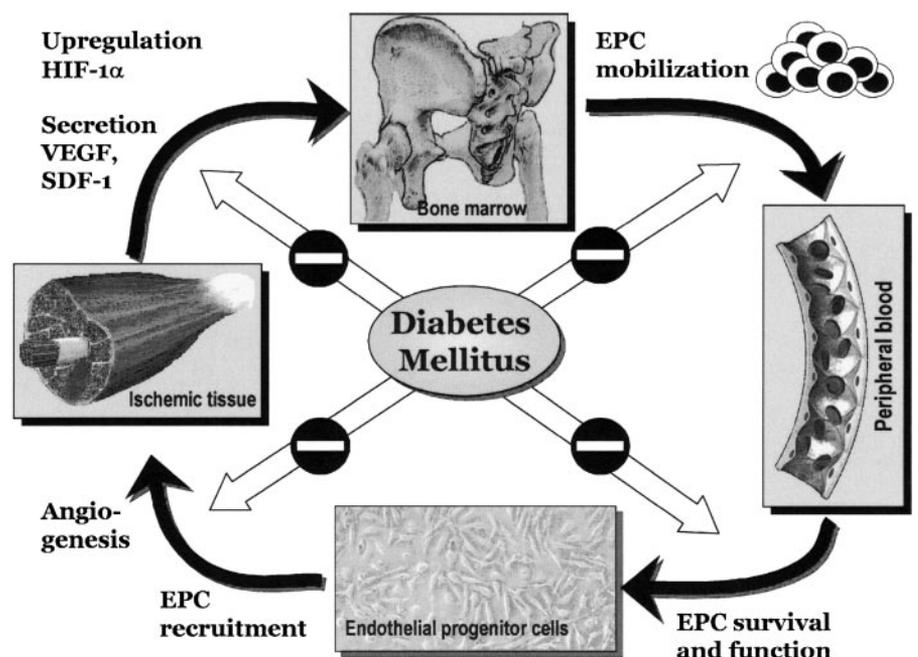

Figure 2—*Diabetes impairs all the steps of EPC mobilization and function.*

deregulation in diabetes depends on an overproduction of reactive oxygen species (ROS). In a recent study (21), ROS inhibition was able to normalize postischemic neovascularization in diabetes by positive EPC modulation. Insulin treatment to achieve normoglycemia during ischemia and reperfusion partially restored the ability to mobilize EPCs through upregulation of growth factors. Consistently, Humpert et al. (22) have shown that insulin therapy in decompensated diabetes increased $CD34^+CD133^+$ progenitor cell count, depending on SDF-1 genotype. Given the positive modulation of EPCs achieved by blood glucose lowering, it is tempting to speculate that favorable clinical outcomes associated with glycemic control during acute ischemic syndromes (23) may be partly dependent on stimulation of EPC-mediated neovascularization in the ischemic myocardium, thus reducing residual ischemia.

We have also shown that diabetic bone marrow is less responsive to exogenous EPC-mobilizing agents. Although molecular mechanisms that regulate EPC release in peripheral blood are complex and not fully understood, a role for the phosphatidylinositol (PI) 3-kinase/protein kinase-B and endothelial nitric oxide (NO) synthase pathways has been shown (24,25). As diabetes is characterized by altered activation of PI 3-kinase/Akt pathways and by reduced NO bioavailability (26), dysfunction of these subcellular pathways may be involved in the defective mobilization of EPCs from bone marrow.

Hyperglycemia may be the common feature that affects survival and function of EPCs because similar alterations have been demonstrated in both type 1 and type 2 diabetes. In vivo, hyperglycemia induces oxidative stress by increasing the production of ROS and alters leukocyte and endothelial function (3). We have previously reported that hyperglycemia activates mitogen-activated protein kinases and protein kinase-C in human circulating peripheral blood mononuclear cells (PBMCs) in vivo (27,28). Recently, Krankel et al. (29) have convincingly demonstrated that high glucose impairs proliferation, survival, and function of cultured EPCs, with concomitant-decreased NO production and matrix metalloproteinase-9 activity. Furthermore, activation of mitogen-activated protein kinases has been revealed as a potential mechanism of EPC dysfunction induced by high glucose

(30). A definite demonstration is that correction of hyperglycemia by insulin therapy (19,22) can indeed restore the normal EPC pool.

Another possible link between diabetes and EPC alterations is the binomial insulin resistance/hyperinsulinemia. In one study, insulin supplementation reduced long-term generation of endothelial cells from $CD34^+$ cells in culture (31). Moreover, we have shown that patients with the metabolic syndrome have decreased levels of $CD34^+KDR^+$ EPCs compared with patients without the syndrome (12). Circulating $CD34^+$ cells are synergically decreased by clustering components of the metabolic syndrome, and their levels negatively correlate with the homeostasis model assessment value, a measure of insulin resistance (32). In fact, insulin resistance, the typical hallmark of metabolic syndrome, is characterized by a defective activation of the PI 3-kinase/Akt pathway and decreased endothelial NO synthase activity, which are considered essential for EPC mobilization and function.

Oxidative stress plays a crucial role in the pathogenesis of diabetes complications (3), as well as in the entire atherogenic process. Therefore, stress-induced apoptosis may be one mechanism of EPC reduction in diabetes. The literature provides ample evidence that EPCs might decrease because of increased apoptosis and that EPCs are stress sensitive (33). For example, estrogens, statins, and physical exercise increase cultured EPCs by inhibiting apoptosis, while C-reactive protein and systemic hypoxia downregulate EPCs by enhancing apoptosis (34–37). Some other works have demonstrated that EPCs display a gene expression profile that confers resistance to oxidative stress (38) and may be related to their ability to survive in hypoxic environments such as ischemic tissues. However, vascular wall cells are directly exposed to systemic oxidative stress, and long-lasting hyperglycemia may downregulate scavenging mechanisms and promote EPC apoptosis, as demonstrated in vitro (29). Moreover, in vivo prooxidant conditions may affect other cells involved in the complex cellular network of the bloodstream and in the vessel wall, which interact with EPCs to determine their function and fate. For instance, in endothelium exposed to vasculature-damaging agents, several enzymes that can produce ROS are upregulated. We have shown that gene expression of NAD(P)H oxidase, a major vascular source of ROS, is increased in circulating

PBMCs from type 2 diabetic patients, depending on glycemic control (39). Remembering that EPCs are a subset of and partly derive from PBMCs, it is easy to imagine how the hostile vascular environment of diabetic patients may negatively influence EPC proliferation, differentiation, and function.

In summary, reduction in circulating EPCs in diabetic patients may recognize at least three pathophysiological explanations: impaired bone marrow mobilization, defective proliferation, and enhanced apoptosis. Remarkably, in accordance with Brownlee's unifying hypothesis (3), oxidative stress appears as a major determinant of all of these mechanisms. Interestingly, two very recent studies have demonstrated that the natural transketolase activator, benfotiamine, which is theoretically able to prevent the subcellular damage pathways triggered by oxidative stress, restored EPC-mediated healing of ischemic diabetic limbs in mice (40) and prevented hyperglycemia-mediated EPC dysfunction via modulation of the Akt pathway (41).

EPCs and diabetic vasculopathy

Accelerated atherosclerosis is probably the most devastating among diabetes complications. The atherogenetic process in diabetic subjects is similar to that observed in their nondiabetic counterparts. However, diabetic vasculopathy is characterized by high prevalence, early development, bilaterality, rapid progression, and typical involvement of multiple distal sites. The severity of macrovascular complications in diabetes has been attributed to a profoundly impaired collateralization of vascular ischemic beds (42), which is insufficient to overcome the loss of blood flow, and leads to critical limb ischemia that often requires amputation. The mechanisms that hinder ischemia-induced neovascularization in diabetes had remained elusive until the discovery of EPCs. In animal models of diabetic vasculopathy, defective collateralization was counteracted by administration of EPCs from control animals. Conversely, diabetic EPCs were not able to stimulate vascularization, even becoming antiangiogenic (31,43). Additionally, EPCs appeared important for vascularization and healing of diabetic wounds (44). Replacement of the diabetic EPCs with a healthy cell pool was an ideal experiment to define their role in the pathogenesis of diabetic vasculopathy, which represented the basis for an in-depth evaluation in humans.

To translate experimental animal data into the clinical setting, we first evaluated the levels of circulating EPCs in patients with and without diabetes and peripheral arterial disease (PAD). In compliance with the observations that diabetes is a cardiovascular disease equivalent, we showed that patients with PAD alone and patients with uncomplicated diabetes had similar EPC reduction versus control subjects. Patients with diabetes and PAD had a further significant decrease in circulating EPC levels, especially in the presence of ischemic foot lesions. Remarkably, EPC levels strongly correlated with the ankle-brachial index, the most objective diagnostic and prognostic test for lower extremity arterial disease (12). Subsequently, we have demonstrated that the EPC decrease in diabetes is closely correlated with the severity of both carotid and lower-limb atherosclerosis: higher degrees of carotid stenosis, as well as worse stages of leg claudication and ischemic lesions, were associated with lower levels of EPCs, suggesting that EPC count may be considered a valuable marker of atherosclerotic involvement. Indeed, cytometric techniques, which allow EPC count, are widely used for routine laboratory testing, and the determination of EPCs is sufficiently reproducible to be used in the clinical practice (9,10,32). Moreover, we have estimated the cost for a single EPC count to be relatively low (~30€ or $40 [U.S.]) in the case that EPCs are defined as $CD34^+KDR^+$ cells. The clinical usefulness would stand in that EPCs not only mirror vascular function and atherosclerotic burden but also reflect the endogenous vasculoregenerative potential. Importantly, there are data suggesting that measuring EPCs would provide additional information over the classical risk factor analysis; in one study (9), $CD34^+KDR^+$ EPC count predicted cardiovascular events independently of risk factors and hard indexes, such as left ventricular ejection fraction.

Moreover, EPCs isolated from diabetic patients with PAD exhibited impaired proliferation and adhesion to mature endothelium (45). We suggest that impaired collateralization leading to the clinical manifestations and complications of atherosclerosis in diabetes may be attributable to decreased and dysfunctional EPCs. Concurrently, increased carotid plaque formation may be related to the depleted reservoir of EPCs, which fails to successfully replace the damaged endothelium. In this light, ways to increase the number and improve the function of EPCs should be actively pursued. A practical consequence of this has been shown: Huang et al. (46) have transplanted bone marrow–mobilized cells, as an EPC-enriched fraction, into critically ischemic limbs of diabetic patients. Compared with standard therapy, cell therapy improved angiographic scores and ankle-brachial index values and reduced relevant end points, such as ulcer size and need for limb amputation. The aggressiveness and distribution of atherosclerosis in diabetic patients often discourages surgical and endovascular revascularization, thus leaving a myriad of no-option patients for whom standard therapies are insufficient. Even if many aspects of cellular revascularization remain to be defined, in the future this novel therapeutic approach may offer a chance for those patients.

EPCs and diabetic cardiomyopathy

Diabetes predisposes to heart failure, and one of its major complications is the development of cardiomyopathy. Contractile depression begins quite early after induction of diabetes in animals, and exercise-induced left ventricular dysfunction is often the first manifestation of cardiac involvement in diabetic patients. Analysis of the left ventricular afterload-pump function relationship reveals that defective contractile recruitment is the main cause of this anomaly (47), which is probably related to an insufficient increase in myocardial perfusion (48). Microvascular abnormalities in the diabetic myocardium include arteriolar pathologies, microaneurysms, and interstitial fibrosis, while the classical underlying metabolic mechanisms include diminished glucose and lactate oxidation paralleled by increased use of fatty acids (49). Recently, Yoon et al. (50) have demonstrated that diabetic cardiomyopathy in rats is characterized by an early and progressive decline in myocardial VEGF expression, which reduces capillary density, increases fibrosis, and impairs contractility. Interestingly, rats with diabetic cardiomyopathy had reduced EPC levels, while restoration of myocardial VEGF expression replenished the circulating EPC pool, which contributed significantly to reconstitute myocardial microvasculature. Taken together, these data suggest that diabetic cardiomyopathy is a complex microvascular complication in which EPC deficiency may be one leading cause of the microcirculatory rarefaction that critically reduces myocardial perfusion. In addition to dysfunction of EPCs, oxidative stress-induced senescence of cardiomyocyte progenitors has been recognized as another potential underlying mechanism in diabetic cardiomyopathy (51). Interestingly, cardiac stem cell aging and heart failure associated with diabetes can be prevented by deletion of the stress-related gene p66Shc, which we have shown to be potently upregulated in type 2 diabetic subjects (52). Prevention and treatment of diabetic cardiomyopathy is a challenge for diabetologists, and the discovery that alterations of circulating and/or local progenitor cells may mediate this complication could identify novel therapeutic strategies.

EPCs and diabetic nephropathy

Diabetes is one leading cause of chronic kidney disease (CKD) in Western countries, and, in turn, CKD is associated with an increased prevalence of cardiovascular risk factors. Therefore, it is not surprising that CKD patients have qualitative and quantitative EPC alterations (53). Mechanisms are largely unknown, but uremic toxins may be involved, as both hemodyalisis and kidney transplantation can restore the endogenous EPC pool (54,55). To date, no study has specifically addressed the question of whether nephropathy per se is associated with further EPC reduction and/or impairment in diabetes, but ~50% of CKD patients enrolled in EPC studies had diabetes.

Endothelial damage and microcirculatory impairment is an early pathogenetic event in diabetic nephropathy (56) and may partly depend on EPC defects. Moreover, some authors have suggested that EPCs are pluripotent and retain the ability to transdifferentiate into disparate phenotypes. Bone marrow–derived cells sharing key markers in common with EPCs have been shown to take part in kidney ontogenesis and regeneration (57), whereas we have shown that the kidney may harbor EPCs under specific conditions (58). Taken together, these data suggest that EPC decline may be one mechanism of defective glomerular repair and renal disease progression in diabetes. In parallel to decrease in endothelial precursors, diabetes causes increase in myofibroblast progenitor cells, which, due to secretion of extracellular matrix components, may contribute to the progressive glomerular obliteration and fibrosis (59).

The relationships between renal function and EPCs are more complex because the kidney-derived hormone erythropoi-

etin has emerged as a predominant regulator of EPC mobilization and differentiation (60). It has been pointed out that the oxygen-erythropoietin feedback, which depends on the hypoxia-sensing system HIF-1α, is dysregulated in diabetes; microangiopathy and progressive tubulointerstitial fibrosis increase the latency of the erythropoietin system, while production of ROS and hyperglycemia itself stabilize HIF-1α, blunting erythropoietin response (61). Additionally, we have recently demonstrated that EPC mobilization in diabetes is defective because of HIF-1α downregulation (19). Another link between nephropathy and altered EPCs is represented by the endogenous NO inhibitor asymmetric dimethylarginine (ADMA), which accumulates in patients with CKD (62) and is elevated in the presence of diabetes and its complications (63). According to the major role played by NO in EPC function (25), ADMA has been recognized as a potent inhibitor of EPC mobilization and function (64). This model depicts a vicious circle in which the EPC alterations associated with diabetes impair renal microvasculature, and, in turn, CKD hampers EPC mobilization, differentiation, and homing through a disrupted erythropoietin system and an excess of ADMA. For these reasons, diabetic nephropathy may be associated with a more profound EPC impairment than CKD in general, which would represent an incremental risk of cardiovascular disease and death.

EPCs and diabetic retinopathy

High blood glucose is an extremely detrimental factor for the retinal microvasculature. Hyperglycemic damage results in increased permeability, blood and serum leakage to the extravascular space, and progressive decline in retinal blood flow. Retinal ischemia and release of angiogenic factors stimulate the proliferation of microvessels, leading to proliferative retinopathy. Recently, intriguing novelties have been added to this pathogenetic model. In animals, bone marrow–derived cells are mobilized and recruited at sites of retinal neovascularization in response to VEGF and SDF-1 (65,66). Therefore, not only local endothelial cells, but also EPCs may be involved in the development of proliferative retinopathy. This seems counterintuitive, as diabetes complications that may affect the same patient, such as diabetic retinopathy and PAD, can have opposing EPC alterations, the one being associated with increased and the

other with decreased EPC levels. Parallely, a single diabetic patient can present at the same time with complications of excessive angiogenesis (proliferative diabetic retinopathy) and of poor angiogenesis (symptomatic PAD). To explore this so-called "diabetic angiogenic paradox," we have studied in vivo the levels of CD34$^+$ and CD34$^+$K diabetic retinopathy$^+$ EPCs and in vitro differentiation of EPCs from type 2 diabetic patients with various combinations of PAD and diabetic retinopathy (67). While CD34$^+$KDR$^+$ cells and endothelial differentiation of cultured progenitors were selectively reduced in PAD patients, generic CD34$^+$ progenitors were reduced in diabetic retinopathy patients, which showed instead higher clonogenic potential and enhanced endothelial differentiation in culture. Almost simultaneously, Asnaghi et al. (68) demonstrated that EPCs cultured from peripheral blood of patients with type 1 diabetes and proliferative diabetic retinopathy displayed increased clonogenic potential. Taken together, these data strengthen the hypothesis that there is a role for EPCs in the development of human proliferative diabetic retinopathy, shifting the pathogenic paradigm from a local environment (retinal) to a systemic one (peripheral blood). Interestingly, pericyte loss is an early and selective event leading to endothelial activation and proliferation in the retina (69), and CD34$^+$ progenitors of perivascular cells have been demonstrated in peripheral blood (70). Thus, according to these notions, depletion of generic CD34$^+$ progenitor cells may cause pericyte loss, whereas increased endothelial differentiation may lead to abnormal retinal angiogenesis. Conversely, depletion of EPCs and reduced endothelial differentiation may hamper collateralization in PAD patients. Therefore, the differential regulation of circulating progenitors, possibly in association with different oxygen gradients and local accumulation of growth factors, may explain why peripheral ischemia cannot stimulate angiogenesis as retinal ischemia does. Going deeper into the systemic events accompanying retinal vascular proliferation may provide novel therapeutic targets against peripheral ischemic complications. The notion that EPCs may be involved in retinal vascular proliferation should induce caution when trying to expand the EPC pool to ameliorate the cardiovascular profile. For instance, erythropoietin itself is an

angiogenic factor that may worsen proliferative diabetic retinopathy (71).

EPCs and diabetic neuropathy

Diabetic neuropathy is caused by both imbalances in neuron metabolism and impaired nerve blood flow. Decrease in vasa nervorum is a prominent characteristic of peripheral nerves in experimental diabetic neuropathy, and decreased blood supply to peripheral nerves can simulate diabetic neuropathy (72). Therefore, maintenance of an adequate network of vasa nervorum is essential to prevent the development of this complication. With this background, EPCs could be important in the homeostasis of the nutritive microvasculture, and their exhaustion or dysfunction may accelerate the course of diabetic neuropathy. Moreover, as progenitor cells derived from the adult blood can be differentiated also toward the neural phenotype (73), it is possible that a broader derangement of immature circulating cells in diabetes predisposes to this chronic complication, dowregulating both endothelial and neuronal progenitors. Unfortunately, no study to date has directly explored these hypotheses. Nevertheless, Naruse et al. (74) have shown that intramuscular administration of EPCs is able to reverse the impairment of sciatic nerve conduction velocity and nerve blood flow in diabetic rats. Once more, the altered EPC regulation in diabetic neuropathy may be attributed to a defective HIF-1α activation (75). Despite the facts that the actual contribution of EPCs to vasa nervorum and nerve function remains to be elucidated and that future studies in humans are needed, it is conceivable that EPC alterations may also be involved in the pathogenesis of diabetic neuropathy. However, it is intriguing that some EPC-modulating agents, such as erythropoietin and statins, have been shown to delay diabetic neuropathy (76).

Therapeutic implications

Given the comprehensive role of EPC alterations in diabetes complications, modulation of the levels and/or function of EPCs may be considered a potential therapeutic strategy. Many drugs provided with beneficial cardiovascular effects, such as statins, ACE inhibitors, and glitazones, have been shown to stimulate EPCs (77,78). However, one may wonder whether diabetic patients, who display a profound impairment in the endogenous EPC pool, are still responsive to those

TABLE 1—*Selection of preclinical and clinical studies on EPCs and diabetes complications*

Complication	Preclinical studies (ref.)	Clinical studies (ref.)
Vasculopathy	Reduced EPC mobilization (19) and EPC-mediated angiogenesis (31)	Reduced EPC number and function in parallel with disease severity (12,45); improvement after EPC transplantation (46)
Cardiomyopathy	Myocardial microvascular rarefaction due to VEGF downregulation and decreased EPCs (50)	None
Retinopathy	Involvement of EPCs in ocular neovascularization (66)	Increased clonogenic potential and differentiation of EPCs (67,68)
Neuropathy	Prevented by EPC administration (74)	None
Nephropathy	Involvement of EPCs in glomerular and tubular repair (57)	EPC decrease in CKD (53) and restoration with dyalisis and transplantation (54,55)
Diabetic foot	Improvement after EPC administration (44)	Wound healing and limb salvage with EPC transplantation (46)

agents. In other terms, what is the best therapeutic intervention in diabetic patients with EPC depression: replacement with administration of ex vivo expanded/ enriched EPCs or pharmacological stimulation of endogenous cells? The present experience indicates that EPC administration is a complex multistep procedure available exclusively at specialized centers during experimental trials. On the other hand, drugs that modulate EPCs in addition to their classic mechanism of action are already widely used to reduce cardiometabolic risk in the general practice so that pharmacological intervention appears safe, easy, widely accessible, and probably effective in diabetic patients as well (79,80). Finally, lifestyle modifications constitute another cornerstone of EPC stimulation because physical exercise has been extensively shown to ameliorate EPC number and function (81,82).

Even if the actual contribution of EPC modulation to the global effect of pleiotropic cardiovascular medications and lifestyle interventions remains unknown, these notions further underline the importance of a multifactorial approach to prevent diabetes complications.

Concluding remarks

According to the novel paradigms of regenerative medicine, bone marrow is a reservoir of immature cells that, once in the bloodstream, participate in regeneration and repair of many tissues thanks to their extreme plasticity. We have discussed the available data demonstrating that decrease or dysfunction of EPCs may have a prominent role in the pathogenesis of all diabetes complications (Table 1 and Fig. 3). Aware that this picture is not complete, we would provokingly suggest that EPCs may represent the fulcrum of a novel unifying theory, which confers a revolutionary centrality to bone marrow, and may also involve adipocyte, fibrocyte, cardiomyocyte, neuronal, and epithelial progenitors acting in concert: it is all in the blood.

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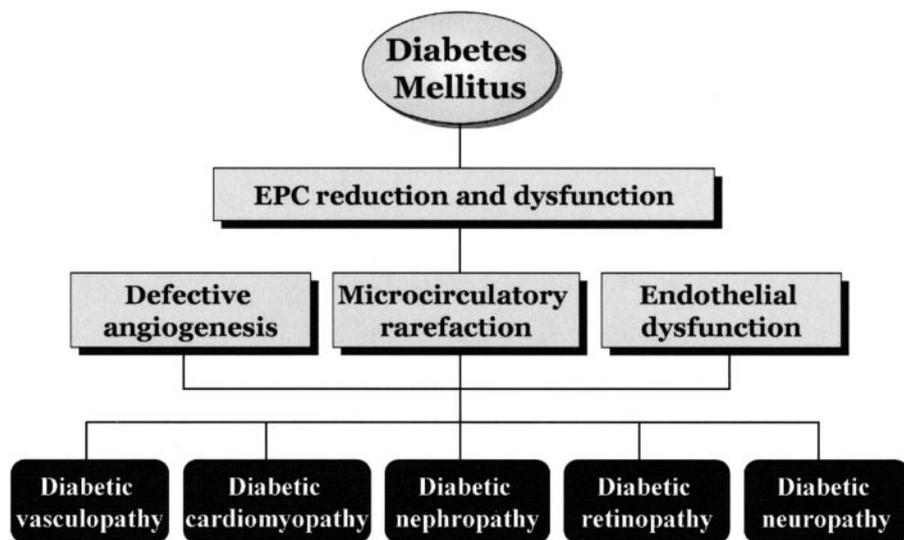
Figure 3—*Diabetes is associated with decrease and dysfunction of EPCs. Subsequently, defective angiogenesis, microcirculatory rarefaction, and endothelial dysfunction are the basis for the development of diabetes complications.*

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