

Impaired Progenitor Cell Activity in Age-Related Endothelial Dysfunction

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OBJECTIVES	We investigated whether human age-related endothelial dysfunction is accompanied by quantitative and qualitative alterations of the endothelial progenitor cell (EPC) pool.
BACKGROUND	Circulating progenitor cells with an endothelial phenotype contribute to the regeneration and repair of the vessel wall. An association between the loss of endothelial integrity and EPC modification may provide a background to study the mechanistic nature of such age-related vascular changes.
METHODS	In 20 old and young healthy individuals (61 ± 2 years and 25 ± 1 year, respectively) without major cardiovascular risk factors, endothelial function, defined by flow-mediated dilation of the brachial artery via ultrasound, as well as the number and function of EPCs isolated from peripheral blood, were determined.
RESULTS	Older subjects had significantly impaired endothelium-dependent dilation of brachial artery (flow-mediated dilation [FMD] $5.2 \pm 0.5\%$ vs. $7.1 \pm 0.6\%$; $p < 0.05$). Endothelium-independent dilation after glycerol trinitrate (GTN) was not different, but the FMD/GTN ratio was significantly lower in old subjects ($49 \pm 4\%$ vs. $37 \pm 3\%$; $p < 0.05$), suggesting endothelial dysfunction. There were no differences in the numbers of circulating EPCs, defined as CD34/KDR or CD133/KDR double-positive cells in peripheral blood. In contrast, lower survival (39 ± 6 cells/mm ² vs. 65 ± 11 cells/mm ² ; $p < 0.05$), migration (80 ± 12 vs. 157 ± 16 cells/mm ² ; $p < 0.01$), and proliferation (0.20 ± 0.04 cpm vs. 0.44 ± 0.07 cpm; $p < 0.05$) implicate functional impairment of EPCs from old subjects. The FMD correlated univariately with EPC migration ($r = 0.52$, $p < 0.05$) and EPC proliferation ($r = 0.49$, $p < 0.05$). Multivariate analysis showed that both functional features represent independent predictors of endothelial function.
CONCLUSIONS	Maintenance of vascular homeostasis by EPCs may be attenuated with age based on functional deficits rather than depletion of CD34/KDR or CD133/KDR cells. (J Am Coll Cardiol 2005;45:1441-8) © 2005 by the American College of Cardiology Foundation

The functional integrity of the endothelial monolayer is essential to prevent vascular leakage in response to local injuries and to protect against the initiation of atherogenesis (1,2). Marked changes in the endothelial morphology include a loss of the orientation of endothelial cells in the

See page 1458

direction of the blood flow, decreased overlap between adjacent endothelial cells, and an accelerated turnover of endothelial cells in response to continuous injury, such as hyperlipidemia, hypertension, and mechanical stress (3). It has been proposed that this kind of disruption of vascular homeostasis predisposes the vessel wall to vasoconstriction, leukocyte adherence, platelet adhesion, thrombosis, vascular inflammation, and eventually atherosclerotic lesion formation.

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Manuscript received July 12, 2004; revised manuscript received November 29, 2004, accepted December 6, 2004.

Among the risk factors for atherosclerosis, aging is a significant predictor of impairment of endothelium-dependent vasodilation (4,5). Experimental and clinical research has demonstrated an increased risk of atherosclerotic disease and poor outcomes in older patients as compared with their younger counterparts (6). Furthermore, it has been suggested that senescent changes in the cardiovascular system may predispose older individuals to increased cardiovascular pathology (7). This suggests that aging is associated with an endogenous alteration of the vessel wall promoting atherosclerosis and vascular dysfunction (8,9).

Although the mechanism for the age-related endothelial dysfunction is not known, the effect of endothelial turnover and regeneration is likely to be involved in the dysfunction (10). Recent studies suggest that besides surrounding mature endothelial cells, bone marrow-derived endothelial progenitor cells (EPCs) may play a critical role in maintaining endothelial function in mature blood vessels by contributing to re-endothelialization and neovascularization (11,12). It is therefore conceivable that any impairment of this vasculogenic element to endothelial regeneration may account for the progression of endothelial dysfunction and atherosclerosis (13).

Abbreviations and Acronyms

- EPC = endothelial progenitor cell
- FACS = fluorescence-activated cell sorting
- FBS = fetal bovine serum
- FMD = flow-mediated dilation
- GTN = glycerol trinitrate
- KDR = kinase insert domain-containing receptor (human VEGF receptor-2)
- MNC = peripheral blood mononuclear cell
- UEA-1 = Ulex europaeus agglutinin I
- VEGF = vascular endothelial growth factor

Extending recent studies showing a reduction of the number and migratory activity in patients with coronary artery disease and a negative correlation of the number of EPC colony-forming units with the Framingham cardiovascular risk factor score (14–17), we focused on the question of whether the sole phenotype aging without apparent cardiovascular disease is associated with a decline in the number and/or function of vascular stem and progenitor cells. We hypothesized that possible age-related alterations in progenitor cell number and function correlate directly with the degree of senescent endothelial dysfunction.

METHODS

Study subjects. We studied 20 young (25 ± 1 year) and 20 old (61 ± 2 years) healthy subjects without clinical evidence of other cardiovascular risk factors. All subjects were asymptomatic, normotensive (as defined by systolic blood pressure <140 mm Hg and diastolic blood pressure <90 mm Hg), nondiabetic (as defined by fasting glucose levels <126 mg/dl), normocholesteremic (as defined by total cholesterol levels <240 mg/dl and low-density lipoprotein [LDL] cholesterol levels <160 mg/dl), and nonsmokers (18–21). They had no significant medical history and were not taking regular or incidental medication, including hormonal contraception. All older female subjects were postmenopausal. No other menstrual cycle history was taken. The clinical characteristics are summarized in Table 1. Informed consent was obtained from all study subjects before enrollment. The

study protocol was approved by the local ethics committee of the Heinrich-Heine University of Düsseldorf.

Determination of endothelial function. Endothelium-dependent dilation of the brachial artery was measured noninvasively by high-resolution ultrasound (Sonos 5500, Agilent, with a 15-MHz linear-array transducer) using standard techniques (22–24). Briefly, baseline data for diameter and mean blood flow velocity of the brachial artery were quantified after 10 min of supine rest in an air-conditioned room (21°C) at 1 to 2 cm above the elbow. To induce ischemic dilation of resistive vessels, a blood pressure cuff was placed around the forearm distal to the cubital fossae and inflated to 200 mm Hg for a period of 5 min. Increased blood flow and shear stress during hyperemia leads to NO-dependent flow-mediated dilation (FMD) of the brachial artery. One minute after deflation of the cuff, the diameter was measured again. Endothelium-independent dilation was quantified 4 min after sublingual administration of 400 µg glycerol trinitrate (GTN; Pohl, Germany). All ultrasound scans were performed by the same operator using the same equipment. An automated analysis system was used to measure diameters (Brachial analyzer, Medical Imaging Applications, Iowa City, Iowa), yielding low variabilities of our methodology described elsewhere (22).

Both FMD and endothelium-independent dilation were expressed as the percent change from baseline. To estimate the relative proportion of FMD compared with the maximally achievable diameter after GTN, the FMD/GTN ratio was calculated and expressed as a percentage for each individual. Blood flow was calculated by multiplication of cross-sectional area and mean velocity at each time point.

Fluorescence-activated cell sorting (FACS) for circulating EPCs. Blood samples were taken on the morning of the ultrasound examination after a 14-h overnight fast to measure the serum lipid profile and other biochemical parameters. Mononuclear cells (MNCs) were isolated from peripheral venous blood by density centrifugation (Histopaque-1077, Sigma-Aldrich, Tuuflurchen, Germany). Freshly isolated MNCs were incubated for 30 min at 4°C in the dark with monoclonal antibodies against human KDR (Sigma-Aldrich), followed by fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse secondary antibody (BD Bio-

Table 1. Baseline Clinical Characteristics of Study Population

Characteristics	Young Subjects (n = 20)	Old Subjects (n = 20)	p Value
Age (yrs)	25 ± 1	61 ± 2	<0.001
Men	10 (50%)	7 (35%)	
Body mass index (kg/m ²)	22 ± 1	24 ± 1	NS
Systolic blood pressure (mm Hg)	117 ± 2	122 ± 3	NS
Diastolic blood pressure (mm Hg)	77 ± 2	81 ± 2	NS
LDL cholesterol (mg/dl)	133 ± 5	142 ± 6	NS
HDL cholesterol (mg/dl)	70 ± 4	72 ± 4	NS
Triglycerides (mg/dl)	87 ± 10	97 ± 8	NS
Glucose (mg/dl)	88 ± 2	91 ± 2	NS
CRP (mg/dl)	0.05 ± 0.03	0.15 ± 0.08	NS

Data are expressed as the mean value ± SE or number (%) of subjects.

CRP = C-reactive protein; HDL and LDL = high- and low-density lipoprotein, respectively; NS = not significant.

sciences, Franklin Lakes, New Jersey), with FITC- or phycoerythrin-conjugated antibodies against human CD45 and CD34 (Beckman-Coulter, Krefeld, Germany) and CD133 (Miltenyi Biotech, Bergisch Gladbach, Germany). Isotype-identical antibodies served as controls (BD Biosciences). After incubation, cells were fixed with 1% paraformaldehyde, and quantitative analysis was performed on EPICS XL (Beckman Coulter) measuring 50,000 cells per sample.

Cell culture enrichment of EPCs. The EPC culture was performed as previously described (25). Briefly, MNCs (7.5×10^6) were plated on fibronectin-coated six-well culture dishes and maintained in endothelial cell basal medium-2 (EBM-2, Clonetics, San Diego, California) supplemented with EGM-2 microvascular single aliquots and 5% fetal bovine serum (FBS). Adherent cells were passaged once at day 4, and the endothelial phenotype was confirmed by flow cytometry (KDR, CD31, CD105, CD146) and immunohistochemistry (von Willebrand factor, tie-2) on day 8 adherent cells before measuring EPC migration and proliferation (25,34). A different protocol was used for the survival assay.

Evaluation of EPC survival. The MNCs (1×10^6) were cultured in four-well glass slides in EBM-2 media supplemented with 100 ng/ml vascular endothelial growth factor (VEGF) protein (Chemicon, Temecula, California). After four days, adherent cells of endothelial lineage were identified by the concurrent binding of FITC-conjugated Ulex europaeus agglutinin I (UEA-1, Sigma) and the uptake of DiI-labeled acetylated low-density lipoprotein (acLDL, CellSystems, St. Katharinen, Germany). Two independent investigators evaluated the number of EPCs per mm^2 by counting dual-staining cells in 15 randomly selected high-power fields using an inverted fluorescent microscope (Nikon, Melville, New York).

Proliferation assay. Proliferation was measured by a colorimetric assay that bases on the cleavage of tetrazolium salts (WST-1) by mitochondrial dehydrogenase. After confirming expression of markers for endothelial lineage, culture-enriched EPCs (1×10^4 cells per well) were plated on fibronectin-coated 96-well plates and cultured for 24 h. After another 48 h of starving with EBM-2 media with 0.1% bovine serum albumin (BSA) (Sigma), WST-1 (TaKaRa Bio, Otsu, Japan) was added, and absorbance at 440 nm was measured with an ELISA reader.

Migration assay. The migratory capacity of culture-enriched EPCs expressing endothelial markers was determined using a modified Boyden chamber. A total of 4×10^4 EPCs were resuspended in EBM-2 plus 0.1% BSA and placed in the upper chamber. The lower chamber contained EBM-2 plus 50 ng/ml recombinant VEGF protein. After 6 h of incubation at 37°C , migrated cells on the lower side of the fibronectin/vitronectin-coated filter (pore size $8 \mu\text{m}$, Neuro Probe, Gaithersburg, Maryland) were fixed and stained with Diff-Quick (Dade Behring, Liderbach, Germany). For quantification, cells were counted manually in five random high-power fields.

Plasma VEGF level. The level of VEGF in the blood plasma of young and old subjects was measured by a high-sensitive ELISA assay (R&D Systems, Minneapolis, Minnesota) according to the manufacturer's instructions. Briefly, all VEGF standards and samples were placed into wells coated with antibody specific for human VEGF. In a second step, a biotinylated antibody specific for VEGF was added to the wells. Afterward, the wells were incubated with streptavidin-horseradish peroxidase and stained by incubation with a substrate solution. The absorbance of standards and samples was measured at 450 nm with a microplate reader. All measurements were performed in triplicate.

Statistical analysis. Results are expressed as the mean value \pm SE. Comparisons between groups were analyzed by the Student *t* test. Univariate correlations were calculated using Pearson's coefficient (*r*). To estimate the predictive value of EPC function in explaining variability of endothelial function measured by FMD, a multivariate linear regression analysis was performed, including established parameters known to affect vascular function and progression of atherosclerosis. Standardized coefficients were calculated as a measure for the relative predictive value. Statistical significance was assumed if a null hypothesis could be rejected at $p = 0.05$. All analyses were performed with SPSS 11.0.1 (SPSS Inc., Chicago, Illinois).

RESULTS

Study subjects. The baseline characteristics were within normal limits and not significantly different between old and young subjects (Table 1).

Endothelial dysfunction in aged individuals. Endothelial function measured by FMD was significantly impaired in old compared with young subjects ($5.2 \pm 0.5\%$ vs. $7.1 \pm 0.6\%$, $p < 0.05$) (Fig. 1). Endothelium-independent dilation in response to GTN was not significantly different ($13.7 \pm 0.8\%$ vs. $15.2 \pm 1.1\%$, $p = \text{NS}$). The FMD/GTN ratio was significantly smaller in old subjects ($49 \pm 4\%$ vs. $37 \pm 3\%$, $p < 0.05$), suggesting selective endothelial dysfunction with a preserved dilatory response of vessel wall smooth muscle cells. The baseline diameter of the brachial artery was significantly smaller in young study subjects ($4.2 \pm 0.1 \text{ mm}$ vs. $3.7 \pm 0.2 \text{ mm}$, $p < 0.05$). No significant differences were seen in blood flow at baseline and during hyperemia ($108 \pm 16 \text{ ml/min}$ vs. $118 \pm 19 \text{ ml/min}$ and $708 \pm 66 \text{ ml/min}$ vs. $644 \pm 38 \text{ ml/min}$, each $p = \text{NS}$).

Effect of age on number of circulating EPCs. To investigate whether age as a sole cardiovascular risk factor is associated with quantitative changes in the circulating endothelial progenitor pool, we isolated MNCs from the peripheral blood of 20 young and 20 old healthy volunteers (for patients characteristics, see Table 1) and used FACS analysis to determine the number of EPCs, considered to be characterized by the expression of KDR and CD34, in the peripheral circulation. We also analyzed the expression KDR and CD133 to examine the number of a more

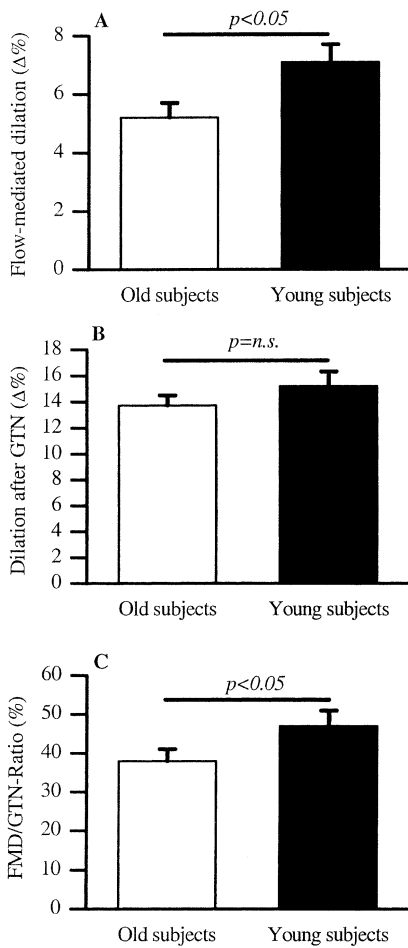


Figure 1. Endothelial dysfunction in old subjects. Whereas (A) flow-mediated dilation (FMD) was significantly impaired in old subjects (n = 20, solid columns), (B) dilatation after 400 μg of sublingual glycerol-trinitrate (GTN) was not (n = 20, open columns). (C) A decreased FMD/GTN ratio in the elder group implies specific endothelial dysfunction. Columns are mean ± SE.

immature subset of progenitor cells. The total numbers per milliliter of blood of both KDR/CD34 double-positive cells and KDR/CD133 double-positive cells were not significantly different between young and old subjects (KDR/CD34+: 436 ± 88 cells/ml vs. 505 ± 88 cells/ml; KDR/CD133+: 373 ± 52 cells/ml vs. 391 ± 68 cells/ml) (Fig. 2). We did not observe a significant difference in the mean fluorescence intensity of the KDR receptor (data not shown).

Because our study population included male and female volunteers, we also analyzed the data for gender-related differences. However, there were no gender differences in progenitor cell numbers within and between both groups (data not shown).

Age-related impairment of EPC survival. To determine the survival and differentiation potential of aged EPCs, we applied a culture assay plating identical numbers of MNCs and cultured the cells in endothelial media using a modified VEGF supplemented protocol. After four days of culture, a reduced number of EPCs, characterized by DiI-acLDL uptake and concomitant UEA-1 binding, were counted

within the old population (39 ± 6 cells/mm² vs. 65 ± 11 cells/mm², p < 0.05) (Fig. 3A).

Functional alterations of EPCs associated with aging. As previously reported, we used culture-enriched EPCs to assess functional differences of progenitors between young and old subjects. The endothelial phenotype was confirmed by demonstrating the expression of KDR (83 ± 8%), CD31 (94 ± 2%), CD105 (75 ± 5%), and CD146 (65 ± 7%) by flow cytometry and by immunocytochemistry for von Willebrand factor and tie-2 (data not shown) (25). We analyzed the proliferatory and migratory capacity of EPCs as important cellular features of progenitor cells (26). The proliferative activity of EPCs was significantly reduced in old subjects (0.20 ± 0.04 vs. 0.44 ± 0.07, p < 0.05) (Fig. 3B). Similarly, the migratory response of EPCs toward a gradient of VEGF was significantly reduced in old subjects as compared their young counterparts (80 ± 12 cells/mm² vs. 157 ± 16 cells/mm², p < 0.01) (Fig. 3C). As implied earlier, this difference was not associated with a reduction of the receptor for VEGF, KDR, on EPCs.

EPC function correlates with endothelium-dependent vascular parameters. We showed a strong univariate correlation between the level of endothelial function and important functional cellular features of progenitor cells. Both the proliferatory activity and the migratory capacity of EPCs correlated significantly with the FMD (r = 0.49, p < 0.05; r = 0.52, p < 0.01) (Figs. 4A and 4B).

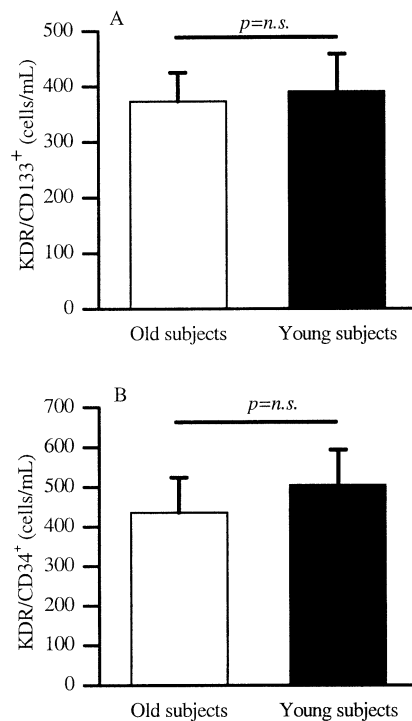


Figure 2. Concentrations of endothelial progenitor cells in old and young subjects. Neither the concentration of (A) KDR/CD133+ or (B) KDR/CD34+ cells was significantly different between old (n = 20, open columns) and young subjects (n = 20, solid columns). Columns represent mean values, error bars are the standard error; p < 0.05.

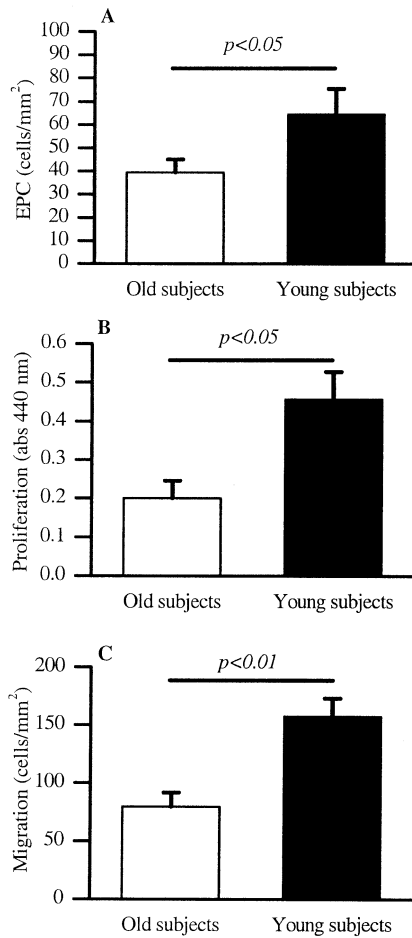


Figure 3. The endothelial progenitor cell (EPC) function in old and young individuals. (A) EPC survival, (B) proliferation, and (C) migration are reduced in old ($n = 20$, open columns) as compared with young study subjects ($n = 20$, solid columns). Columns represent mean values, error bars are the standard error.

Plasma VEGF level. To determine changes in the cytokine or growth factor setting associated with aging, we measured the expression level of VEGF, which is an important regulator of EPC recruitment. The VEGF concentration in the peripheral blood were significantly higher in old as compared with young subjects (93.7 ± 5.6 pg/ml vs. 61.4 ± 3.8 pg/ml, $p < 0.001$).

Independent predictors of endothelial function. In order to identify independent predictors for endothelial function measured by FMD, we performed a multivariate linear regression analysis, including baseline characteristics known to affect vascular function (age, gender, LDL, plasma glucose, C-reactive protein, baseline diameter of brachial artery, VEGF) and parameters of EPC function (survival, migration, proliferation). As shown in Table 2, the only independent predictors for FMD were EPC proliferation, EPC migration, and baseline diameter of brachial artery, accounting for 53% of the total variability of FMD (adjusted $R^2 = 0.528$, $p < 0.001$).

DISCUSSION

Aging stem cells may play a critical role in determining the effects of aging on organ function. With regard to vascular diseases, it has been postulated that circulating EPCs are involved in the repair mechanisms after endothelial damage (27,28). Ultimately, deterioration of endothelial or vascular function may be related to both quantitative and qualitative changes of stem cells.

We describe here the first comprehensive analysis of the association between age-related endothelial dysfunction and the number and function of circulating EPCs, defined by expression of CD34+/VEGFR2+ and CD133+/VEGFR2+. Although no quantitative differences in EPCs were observed, our data illustrate that culture-enriched EPCs from old but otherwise healthy subjects are impaired in terms of fundamental functional features like proliferation (important for amplifying the cellular pool), migration (critical for homing of circulating EPCs), and survival. We demonstrate a significant univariate correlation between the proliferative and migratory capacity of EPCs and FMD. In a multivariate model, both functional features of progenitor cells represent independent predictors of endothelial function, indicating that abnormalities in EPC function may account for the impaired vascular regeneration and repair observed in the old.

Elderly individuals showed endothelium-specific dysfunction of vascular reactivity, indicated by impaired FMD with preserved endothelium-independent dilation, and a significantly lowered FMD/GTN ratio. In order to specifically investigate the effect of aging on EPCs and endothelial function, major cardiovascular risk factors associated with endothelial dysfunction, hypertension, hyperlipidemia, diabetes mellitus, and cigarette smoking were excluded. Plasma glucose, LDL cholesterol, blood pressure, C-reactive protein, and body mass index were not significantly different between old and young subjects and were not independent predictors of FMD in the multivariate analysis. Other than EPC migration and proliferation, the only independent predictor of endothelial function was the baseline diameter of the brachial artery. The greater baseline diameter of the brachial artery, reported to inversely correlate with FMD (29), can therefore at least in part account for a lower FMD in older subjects. In fact, the standardized coefficient for baseline diameter was comparable to the sum of migration and proliferation coefficients indicating a similar magnitude of association.

Changes in stem and progenitor cells may be of great importance for the aging process, because any decline with age in the numbers and functional integrity of stem cells could potentially lead to progressive deterioration of functional and proliferative homeostasis in organs (30). Current explanations for impaired neovascularization or re-endothelialization with age have involved the conventional paradigm of angiogenesis and focused on alterations in mature endothelial cells and monocytes. Senescent changes in endothelial function are accompanied by changes in angiogenic activity and alterations

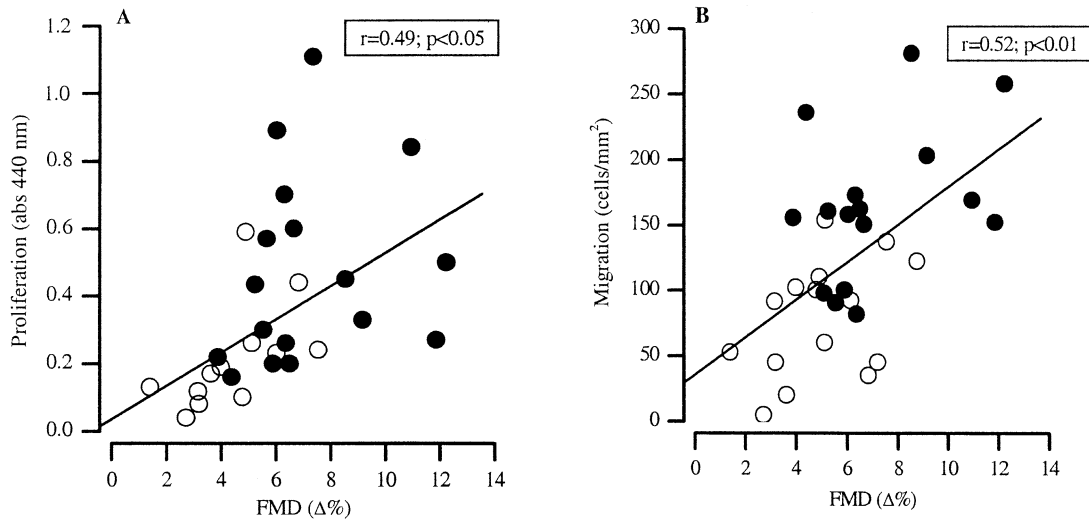


Figure 4. Correlations of endothelial function and endothelial progenitor cell (EPC) function. Flow-mediated dilation (FMD) correlated with (A) EPC proliferation ($r = 0.49$, $p < 0.05$) and (B) migration ($r = 0.52$, $p < 0.01$). **Open circles** = old subjects; **solid circles** = young subjects.

in growth factor expression (10,31), indicating that in the old, local endothelial cells are not capable of reacting to various forms of vessel wall injury in response to excessive and inflammatory/proliferatory processes.

Recent studies support the notion that one plausible mechanism for the vascular changes in advancing aging is the exhaustion of cells, in particular, bone marrow cells that are capable of vascular repair (13,32). Exhaustion of this component of bone marrow in number, function, or both could produce disequilibrium between vascular injury and vascular repair, which leads to atherosclerosis (2,33). Indeed, several studies demonstrated that the number of circulating EPCs inversely correlated with risk factors for coronary artery disease, such as smoking, family history of coronary artery disease, hypertension (34), and diabetes mellitus (35). Interestingly, our study did not reveal a significant difference in the total number of circulating stem cells and the progenitor pool between old and young persons. Rather, we found an increased percentage of CD34+/KDR+ cells in old individuals. However, due to the decrease in the number of mononuclear cells in the older subjects, the total number of circulating stem cells was not significantly different. Together with the data revealing a significantly higher VEGF plasma level and reduced EPC survival in the elderly, this may reflect the attempt of the aged organism to mobilize vascular stem and progenitor cells into the peripheral blood in response to endothelial dysfunction at an early stage of atherosclerosis. It also suggests that greater stimulation is required to maintain the circulating numbers of progenitor cells in the circulation. A decreased number of other cells that are potential sources of putative endothelial cells (e.g., CD34-/CD14+ monocytes) cannot be excluded (36,37).

It is conceivable that aging is associated with dysfunctional progenitor cells and defective vasculogenesis. Preliminary results from animal studies indicated that transplan-

tation of bone marrow (including EPCs) from old mice into young mice led to minimal neovascularization relative to transplantation of young bone marrow (38). It was also demonstrated that EPCs from older patients with clinical ischemia had a significantly less therapeutic effect in rescuing ischemic hindlimb of nude mice compared with those from younger ischemic patients (38,39). These studies provide evidence to support an age-dependent impairment in neovascularization that is heavily influenced by the EPC phenotype. In accordance with these and other data, our results show that aging as a sole cardiovascular risk factor is associated with diminished fundamental functional features of progenitor cells. Thus, beyond impaired EPC mobilization, the activity of progenitor cells in response to tissue injury may contribute to the age-dependent defect in postnatal re-endothelialization and neovascularization. As a critical point, it has to be noted that the results of functional assays are based on ex vivo expanded EPCs and do not

Table 2. Multivariate Linear Regression Analysis

	FMD	
	Standard Coefficient	p Value
Age	0.205	0.242
Gender	-0.308	0.110
LDL	-0.054	0.663
Plasma glucose	-0.145	0.243
CRP	0.078	0.540
Diameter brachial artery	-0.755	0.001
EPC survival	0.043	0.740
EPC migration	0.342	0.015
EPC proliferation	0.429	0.002
VEGF	0.095	0.470
Adjusted R ²	0.528	
Significance (ANOVA)		<0.001

Bold entries indicate significant p values.

ANOVA = analysis of variance; CRP = C-reactive protein; EPC = endothelial progenitor cell; FMD = flow-mediated dilation; LDL = low-density lipoprotein; VEGF = vascular endothelial growth factor.

necessarily reflect the potential of primitive cells present in the human circulation. Other factors (i.e., reduced adhesion) may lead to a different selection of cells during culture.

Recently, Hill et al. (16) described the correlation between the number of EPC colonies after one week of culture, as an equivalent for the level of circulating EPCs, with the brachial artery reactivity as an index of endothelial function. It was concluded that the quantity of EPCs provides a useful index of cumulative cardiovascular risk and vascular function in subjects with various degrees of cardiovascular risk. Focusing on age as an isolated risk factor, we show correlations between endothelial function and EPC functions as measured after culture expansion but not circulating EPC numbers as measured by expression of CD34+/VEGFR2+ and CD133+/VEGFR2+ in peripheral blood. We argue that these results are in line with the findings of Hill et al. The number of EPC colonies is not only dependent on the number of EPCs used for the assay, but most importantly relies on the proliferatory capacity, adhesion, and survival of cells during culture. Therefore, we reason that functional rather than numeric changes of certain vascular progenitor cells may be responsible for age-related conditions. Increased VEGF levels may compensatorily mobilize EPCs, explaining similar EPC numbers despite lower survival. However, multivariate analysis showed that proliferation and migration of EPCs represent independent predictors of endothelial function, indicating a regenerative role of EPCs in early rather than late stages of vascular damage.

The continuous circulation of vascular stem and progenitor cells may provide a homeostatic means of meeting the stem cell crisis in response to stress or organ injury. This kind of vascular repair mechanism is critical for maintaining organ viability in a setting of impaired blood flow or hemostatic disruption. Understanding senescence-associated changes in vascular function, as well as the role that cells with regenerative potential play in modulating vascular reactivity, will be important for the development of more effective therapies for the prevention and treatment of cardiovascular disease in older individuals.

Acknowledgment

The authors thank Simone Matern for technical assistance.

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