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ORIGINAL ARTICLE

Circulating endothelial progenitor cells and depression: a possible novel link between heart and soul

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Although depression is known to be an independent risk factor for cardiovascular disorders, the mechanisms behind this connection are not well understood. However, the reduction in the number of endothelial progenitor cells (EPCs) in patients with cardiovascular risk factors has led us to hypothesize that depression influences the number of EPCs. EPCs labeled with CD34, CD133 and vascular endothelial growth factor receptor-2 (VEGFR2) antibodies were counted by flow cytometry in the peripheral blood (PB) of 33 patients with a current episode of major depression and of 16 control subjects. Mature (CD34+/VEGFR2+) and immature (CD133+/VEGFR2+) EPC counts were decreased in patients (vs controls; $P<0.01$ for both comparisons), and there was a significant inverse relationship between EPC levels and the severity of depressive symptoms ($P<0.01$ for both EPC phenotypes). Additionally, we assayed the plasma levels of VEGF, C-reactive protein (CRP) and tumor necrosis factor (TNF)-α and observed significantly elevated TNF-α concentrations in patients (vs controls; $P<0.05$) and, moreover, a significant inverse correlation between TNF-α and EPC levels ($P<0.05$). Moreover, by means of a quantitative RT-PCR approach, we measured CD34, CD133 and VEGFR2 mRNA levels of PB samples and found a net trend toward a decrease in all the investigated EPC-specific mRNA levels in patients as compared with controls. However, statistical significance was reached only for VEGFR2 and CD133 levels ($P<0.01$ for both markers). This is the first paper that demonstrates evidence of decreased numbers of circulating EPCs in patients with a current episode of major depression.
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Keywords: cardiovascular diseases; mood disorders; depression; endothelial progenitor cells; postnatal vasculogenesis

Introduction

Mood disorders with episodes of major depression (as part of unipolar major depression and bipolar disorder) are frequent illnesses with enormous personal and society burdens worldwide. The life-time prevalence of major depressive and of bipolar disorders are 5–17 and 0.3–7.2%, respectively.[1] It is widely known that major depression or depressive symptoms (as part of dysthymia) are risk factors not only for suicide but also for non-suicide mortality as well.[2–6] Several studies with prospective design—after controlling for possible confounding factors such as hypertension, diabetes mellitus, smoking and age—have concluded that depression predicts the development of cardiovascular diseases.[4,7,8] Accordingly, depression confers a relative risk between 1.5 and 2.0 for the onset of coronary artery disease in physically healthy individuals and a relative risk between 1.5 and 2.5 for cardiac morbidity and mortality in patients with existing coronary artery disease.[9] Moreover, large-scale studies revealed an association between mood disorders and susceptibility to stroke.[10–12] However, although numerous theories have been proposed to explain the amplified risk of cardiovascular disease in patients with depression (reviewed by Everson-Rose and Lewis,[3] Lett *et al.*[9] and Holtzheimer and Nemeroff[13]), the exact biological mechanisms by which depression may increase the risk of cardiovascular events have not been completely elucidated so far.

Adult bone marrow contains a subtype of progenitor cells that has the capacity to migrate to the circulation

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and to incorporate into the endothelial layer of blood vessels. This cell population, endothelial progenitor cells (EPCs), appears to be involved in both the maintenance of vascular integrity[14] and postnatal vasculogenesis (for example, tumor vascularization).[15–18] Since their identification by Asahara *et al.*,[19] several studies have shown reduced numbers and/or impaired function of EPCs in a variety of cardiovascular risk states, including diabetes mellitus,[20] hypercholesterolemia,[21] hypertension,[22] chronic renal failure,[23] rheumatoid arthritis[24] and cigarette smoking.[25] Alternatively, cardiovascular protective factors such as exercise training,[26] statin therapy,[27] angiotensin II receptor antagonists[28] and peroxisome proliferator-activated receptor agonists[29] are known to increase EPC number and function.

Because depression is characterized by increased cardiovascular morbidity and mortality that cannot be explained by traditional cardiovascular risk factors alone and depressive disorders were found to be associated with dysfunction of the immune system and the bone marrow,[30,31] we hypothesized that depression influences the number of bone marrow-derived EPCs as well. Hence, using peripheral blood (PB) samples obtained from healthy individuals and from patients with a current episode of major depression, we assessed the numbers of circulating EPCs by flow cytometry and investigated whether these numbers may be related to the presence and severity of depression. Furthermore, we measured the levels of the key vasculogenic molecule vascular endothelial growth factor (VEGF) and the proinflammatory cytokines tumor necrosis factor (TNF)-α and C-reactive protein (CRP), and used real-time quantitative reverse transcription (RT)-PCR to study the expression of the EPC-specific markers CD34, CD133 and VEGF receptor-2 (VEGFR2) in the PB of depressed patients and healthy controls.

Materials and methods

Clinical data

Thirty-three in- and outpatients diagnosed with a major depressive episode in two psychiatric centers participated in the study (Table 1). The presence of a current major depressive episode was diagnosed by the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) criteria.[32] The nine-item abbreviated version of the Beck Depression Inventory (BDI) was used for the assessment of the severity of depressive symptoms.[33] During participant enrollment, complete clinical (physical examination including blood-pressure monitoring, height and body weight) and laboratory evaluations were carried out (Table 1). Patients with elevated levels of fasting blood-glucose, creatinine, urea nitrogen or liver functions, or with hypertension, comorbid psychiatric diagnoses, BMI higher than 30 $kg\,m^{-2}$, higher cholesterol or triglyceride levels than upper levels of Adult Treatment Panel III[34] defined borderline hypercholesterolemia (240 $mg\,100\,ml^{-1}$ or 6.2 $mmol\,l^{-1}$) and hypertriglyceridemia (200 $mg\,100\,ml^{-1}$ or 2.25 $mmol\,l^{-1}$), or with signs of infection (subfebrile temperature, fever, white blood cell number higher than 10 $g\,l^{-1}$, erythrocyte sedimentation rate higher than 20 $mm\,h^{-1}$) were excluded from the study. Moreover, cardiovascular

Table 1 Baseline clinical characteristics and cytokine levels of patient and control groups

	Patients (n = *33*)	*Controls* (n = *16*)	P-*value*
Gender (female/male)	29/4 (88 vs 12%)	14/2 (88 vs12%)	0.98[†]
Age (years)	40.6 ± 10.6	40.3 ± 9.5	0.93*
BUN ($mmol\,l^{-1}$)	4.26 ± 1.52	5.14 ± 1.18	0.055*
Body mass index ($kg\,m^{-2}$)	23.3 ± 3.49	22.7 ± 4.1	0.61*
White blood cells (10^9 per liter)	7.27 ± 1.83	7.94 ± 1.48	0.18*
Blood glucose (mmol per liter)	4.75 ± 0.58	4.52 ± 0.75	0.31*
Total cholesterol (mmol per liter)	4.69 ± 0.82	5.11 ± 0.93	0.14*
Triglyceride (mmol per liter)	1.17 ± 0.5	0.89 ± 0.57	0.11*
hs-CRP ($mg\,dl^{-1}$)	0.13 ± 0.06	0.11 ± 0.04	0.29*
TNF-α ($pg\,ml^{-1}$)	2.68 ± 0.8	1.5 ± 0.46	0.03*[#]
VEGF ($pg\,ml^{-1}$)	19.37 ± 3.83	17.35 ± 3.82	0.1
BDI score	38.6 ± 10.7	0.9 ± 1.44	< 0.01[§#]
Smoking status (current smoker/ nonsmoker)	19/14 (58 vs 42%)	10/6 (62.5 vs 37.5%)	0.74[‖]
Smoking amount in smoker subgroups (no. of cigarettes per day)	23.1 ± 11.7	15.3 ± 9.3	0.064*

Abbreviations: BDI, beck depression inventory; BUN, blood urea nitrogen; hs-CRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor.
Data are expressed as mean + s.d.
*Independent-samples *t*-test
[†]Fischer's exact test.
[§]Mann–Whitney's test.
[‖]χ^2-test.
[#]Significant difference between patient and control groups.

risk factors (diabetes mellitus, hypertension, hypercholesterolemia, hypertriglyceridemia, renal failure) and cardiovascular diseases (acute myocardial infarction, arterial obstructive syndromes of limbs, ischemic stroke) in the medical history of the patients were also criteria for exclusion. Accordingly, no participants received medications with known effects on EPC numbers (Table 2). Data about the smoking habits (smoking status and intensity, defined as self-reported average number of cigarettes smoked daily) of patients and healthy controls were collected as well (Table 1). Based on smoking behavior, the following categories were used: 'non-smokers' (ex- and never smokers) and 'current smokers.' Based on the observations of Kondo *et al.*[25] on the effects of smoking cessation on EPC levels, ex-smokers were defined as those who had quit smoking at least 1 month before taking the blood sample.

The control group included 16 individuals matched for age, gender and smoking status (Table 1). Smoker and non-smoker subgroups of patients and controls were also matched for age. Exclusion criteria for control persons were the same as those for patients with depression. Healthy controls had no previous or current episode(s) of major depression. The study was approved by the Local Ethical Committees of the National Institute of Psychiatry and Neurology, Budapest, and of the Central Hospital of the Hungarian Army, Budapest. All subjects gave their informed consent.

Table 2 Medications of patients and control persons

Psychotropics	*No. of patients receiving*
Anxiolytics[a]	29
Second-generation antipsychotics	4
Mood stabilizers	10
Hypnotics	8
SSRIs	19
SNRIs	7
Other antidepressants	13
Other medications	*No. of patients (P) or control persons (C) receiving*
Tiotropium bromide	1 (P)
Calcitonin	1 (P)
Piroxicam	1 (P)
Propranolol[b]	2 (P)
L-Thyroxine	1 (P)
Calcium dobesilate	1 (P)
Diclofenac	1 (C)

Abbreviations: SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.
No participants received medications with known effects on EPCs.
[a]Benzodiazepines were given to all except one patient, who received hydroxyzine hydrochloride.
[b]For tachycardia.

Enumeration of EPCs by flow cytometry from the peripheral blood of controls and patients with depression

To quantify the content of circulating EPCs by flow cytometric analysis, following erythrocyte lysis, the remaining PB mononuclear cell fraction was resuspended in 90 µl of a fluorescence-activated cell-sorting buffer containing phosphate-buffered saline and 0.1% bovine albumin and incubated for 30 min at 4 °C with R-Phycoerythrin (PE)-Cy5-conjugated anti-human CD34 (BD Biosciences, San Jose, CA, USA) and allophycocyanin (APC)-conjugated anti-human VEGFR2 (R&D Systems, Minneapolis, MN, USA) or with PE-conjugated anti-human CD133 (Miltenyi Biotec, Bergisch Gladbach, Germany) and APC-conjugated anti-human VEGFR2. Appropriate fluorochrom-conjugated isotype controls were used for each staining procedure. After appropriate gating, the number of CD34+/VEGFR2+ and CD133+/VEGFR2+ cells were quantified and expressed as the number of cells per milliliter of blood using the CyFlow SL flow cytometer and the FlowMax software (both from Partec, Münster, Germany).

Measuring the level of EPC markers by quantitative real-time RT-PCR in the peripheral blood of controls and patients with depression

PB was incubated for 10 min with Red Blood Cell Lysing Buffer (Sigma-Aldrich, Budapest, Hungary), and centrifuged for 20 s at full speed in a microcentrifuge. Total RNA was extracted from the remaining PB mononuclear cell fraction after lysis using Qiagen RNeasy Mini Kit (Hilden, Germany) and digested with RNase-free DNase Set according to the manufacturer's protocol. Total RNA (3 µg) was reverse transcribed from each sample using deoxy-NTPs (0.5 mM each), a mixture of random primer and oligo dT (final concentration 3 µM), RNasin ribonuclease inhibitor (20 U per reaction, Promega, Madison, WI, USA), reverse transcriptase buffer and M-MLV reverse transcriptase (200 U per reaction, Sigma-Aldrich). Samples (30 µl) were incubated for 50 min at 37 °C and then at 85 °C for 10 min. The sequences of CD34 primers[35] were 5′-TTGACAACAACGGTACTGC TAC-3′ and 5′-TGGTGAACACTGTGCTGATTAC-3′. The sequences of CD133 primers[35] were 5′-TGGATGC AGAACTTGACAACGT-3′ and 5′-ATACCTGCTACGA CAGTCGTGGT-3′. The sequences of VEGFR2 primers[35] were 5′-CACCACTCAAACGCTGACATGTA-3′ and 5′-GCTCGTTGGCGCACTCTT-3′. The real-time PCR analysis was standardized by co-amplifying the genes of interest with the housekeeping gene β-actin (primers: 5′-TCTGGCACCACACCTTCTAC-3′ and 5′-CTCC TTAATGTCACGCACGATTTC-3′). The real-time PCR reaction was run on the iCycler iQ (Bio-Rad, Richmond, CA, USA) using standard conditions, namely, an optimized concentration of primers (final concentration 200 nM), iQ SYBR Green Supermix and 2 µl cDNA. A no-template control (containing water) was used as a negative control for every different primer-pair. The cycling parameters were 95 °C

(3 min), 50 cycles of 95 °C (30 s), 64 °C (30 s) and 72 °C (1 min). The starting quantity of gene expression in the sample was determined by comparison of an unknown to a standard curve generated from a dilution series of template DNA of known concentration, and normalized to its own β-actin expression.

Measuring the levels of CRP, VEGF and TNF-α in the peripheral blood of controls and patients with depression

For CRP, VEGF and TNF-α measurements, plasma samples from all patients and controls were prepared and stored at −80 °C until further analysis. Levels of VEGF and TNF-α were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (both from R&D Systems) according to the manufacturer's instructions. Results were compared with standard curves, and the lower detection limits were VEGF, 5 pg ml^{-1}; TNF-α, 0.2 pg ml^{-1}. Measurements were performed in duplicate. Concentrations of CRP were determined by turbidimetric immunoassay (Olympus CRP Latex assay, Hamburg, Germany).

Statistical analysis

Continuous variables were compared with Student's *t*-test. The differences among more than two groups were analyzed with analysis of variance (ANOVA) and Scheffe's *post hoc* method. Continuous data were compared with Mann–Whitney *U*-test if the sample distribution was asymmetrical. Categorical data were compared using Fishers' exact probability and χ^2-tests. Linear regressions were analyzed using the simple regression model. Correlations of EPC and cytokine levels were determined using Spearman's rank correlation test. Differences were considered significant when $P < 0.05$. All statistical analyses were carried out using Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA) software program.

Results

Characterization and numbers of EPCs in peripheral blood samples of controls and patients with depression

EPCs are thought to derive from CD34+ hematopoietic progenitor cells and can be identified by the expression of the cell surface markers CD34, CD133 and VEGFR2.[36] However, these surface molecules are differentially expressed in late/mature and in early/immature phenotypes and none of them by itself is specific for EPCs. The lack of a special and exclusive marker truly specific for EPCs dictates that combinations of the above markers must be used to best identify this cell population.[14,18,37–39] Therefore, we determined the numbers of both CD34+/VEGFR2+ (late/mature phenotype; Figure 1a, cells in Q2) and CD133+/VEGFR2+ (early/immature phenotype; Figure 1b, cells in Q2) double-positive EPCs in the PB of healthy individuals and depressed patients by flow cytometry. By the same token, cell populations positive for only one marker (Figures 1a and b, cells in Q1 or Q4) were not considered in our experiments.

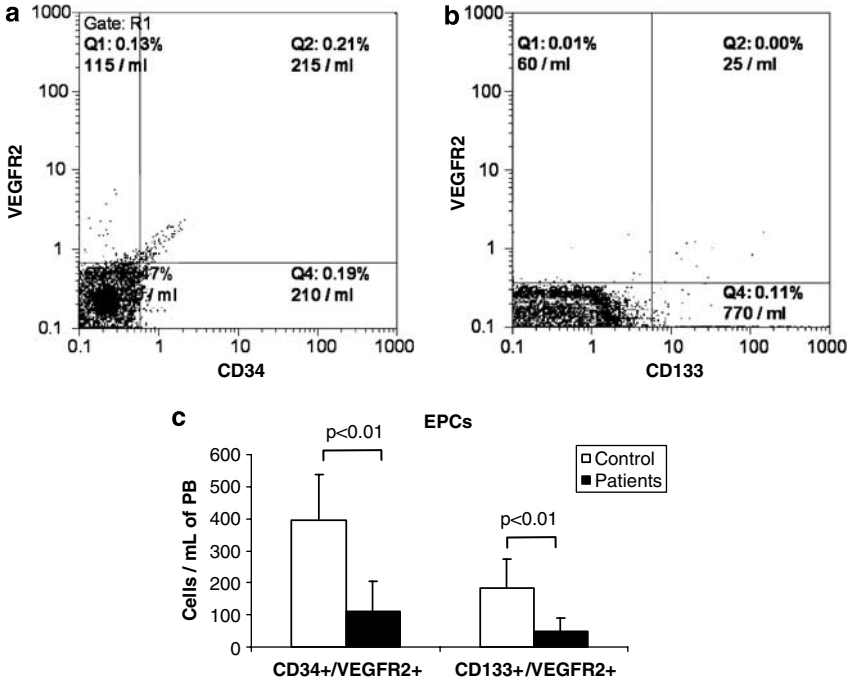

Figure 1 Quantitative evaluation of circulating endothelial progenitor cells (EPCs) by flow cytometric analysis. (**a**, **b**) Representative flow cytometric analyses for determining the number of CD34+/ vascular endothelial growth factor receptor-2 (VEGFR2+) (**a**) and CD133+/VEGFR2+ (**b**) double-positive cells. (**c**) Circulating EPC levels in healthy controls ($n = 16$) and patients with depression ($n = 33$). Data are mean EPC numbers per milliliter of peripheral blood (PB) ± s.d. In (**a**), Q1 = CD34−/VEGFR2+, Q2 = CD34+/VEGFR2+, Q3 = CD34−/VEGFR2−, Q4 = CD34+/VEGFR2− cells. In (**b**), Q1 = CD133−/VEGFR2+, Q2 = CD133+/VEGFR2+, Q3 = CD133−/VEGFR2−, Q4 = CD133+/VEGFR2− cells.

In the patient population, both the levels of mature (CD34+/VEGFR2+) and immature (CD133+/VEGFR2+) EPCs per milliliter of PB were significantly lower than those in the group of healthy controls ($P<0.01$ for both comparisons; Figure 1c). There was no statistically significant correlation between age and EPC levels either in the patient or in the control group (data not shown). Nevertheless, it is important to note that the patient and control populations investigated in the current study included mostly middle-aged individuals (Table 1). EPC numbers of our participants were also evaluated according to the severity of depressive symptoms (as assessed by the BDI[33]). There was a statistically significant inverse relationship between EPC levels and BDI scores ($P<0.01$ in cases of both EPC phenotypes, data not shown) independent of the phenotypes of EPCs.

Because cigarette smoking has recently been demonstrated to decrease circulating EPC numbers,[25,40] patients and controls were matched for smoking habits, as shown in Table 1. In addition, smoker and non-smoker subgroups of patient and control populations were also matched for age. In smoker subgroups, the mean ages of patients and controls were 39.5 ± 11.1 years and 39.7 ± 7.71 years, respectively (mean ± s.d.; $P=0.95$). In non-smoker subgroups, the mean ages of patients and controls were 42.1 ± 10.2 years and 41.3 ± 12.65 years, respectively (mean ± s.d.; $P=0.9$).

Assessment of EPC numbers using CD34/VEGFR2 labeling indicated a significant decrease among smokers compared to non-smokers in both control and patient groups (P-values are <0.01 and <0.001, respectively; Figure 2a). Quantification of EPCs by CD133/VEGFR2 labeling also revealed that the EPC level in the control population was significantly lower in smokers as compared with non-smokers ($P<0.01$; Figure 2b). Although a similar tendency was observed, the difference in CD133+/VEGFR2+ EPC levels between smokers and non-smokers remained statistically insignificant in the patient population ($P=0.34$; Figure 2b). When smoker controls were compared with smoker patients and non-smoker controls with non-smoker patients, both the CD34+/VEGFR2+ (Figure 2a) and the CD133+/VEGFR2+ (Figure 2b) EPC levels were significantly lower in the patient groups.

Evaluation of EPC markers in peripheral blood samples of controls and patients with depression by quantitative real-time RT-PCR

CD34, CD133 and VEGFR2 mRNA levels in healthy controls and in 33 patients with major depression were determined by quantitative real-time RT-PCR (Figure 3). Levels of VEGFR2 and CD133 were significantly lower in the PB of patients when compared with the levels of healthy controls ($P<0.01$ for both comparisons, (Figures 3b and c), whereas CD34 level was not significantly decreased in patients with depression ($P=0.08$, Figure 3a). Accordingly,

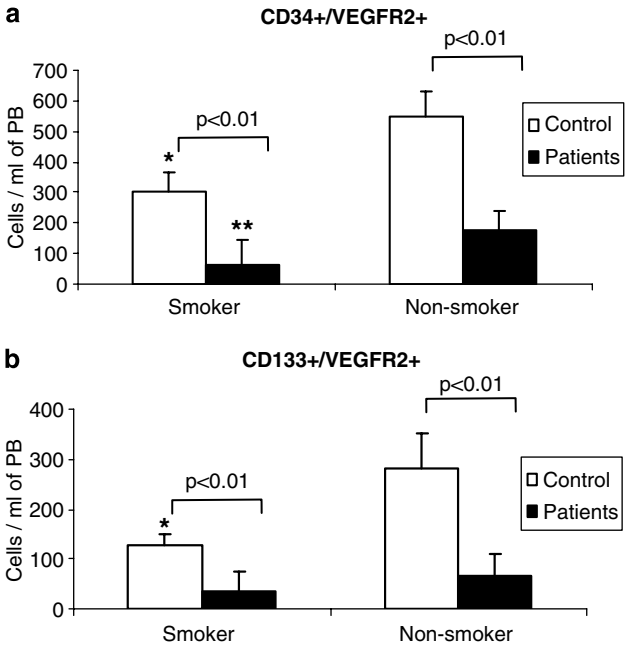

Figure 2 Quantitative evaluation of circulating endothelial progenitor cells (EPCs) by flow cytometric analysis based on both CD34+/vascular endothelial growth factor receptor-2 (VEGFR2+) (**a**, **b**) CD133+/VEGFR2+ double labeling in smoker and non-smoker subgroups of controls and patients. Data are mean EPC numbers per milliliter of peripheral blood (PB) ± s.d. In (**a**), the single asterisk (*) marks significant difference ($P<0.01$) between smoker and non-smoker controls. The double asterisks (**) mark significant difference ($P<0.001$) between smoker and non-smoker patients. In (**b**), the single asterisk (*) marks significant difference ($P<0.01$) between smoker and non-smoker controls.

although there was a statistically significant inverse relationship between VEGFR2 and CD133 mRNA levels and BDI scores ($P<0.01$ in cases of both markers, data not shown), no such correlation was present in the case of CD34. Furthermore, as in the results of flow cytometric analyses, there was no statistically significant association between age and EPC marker levels either in the patient or in the control group (data not shown). Finally, although we also investigated the relationship between EPC numbers and the levels of EPC-specific mRNA levels, statistically significant results were found only in the case of CD133 ($P<0.01$ in cases of both mature and immature EPC phenotypes, data not shown).

When subjects in the control and the patient groups were classified according to their smoking status (Table 1), no significant differences in CD34, CD133 and VEGFR2 mRNA levels between smokers and non-smokers were demonstrated, either within the control or within the patient population ($P>0.05$ for all analyses, Figure 4).

Comparisons of both smoker controls to smoker patients and non-smoker controls to non-smoker patients with respect to all the investigated EPC markers showed higher levels of mRNA in the PB of

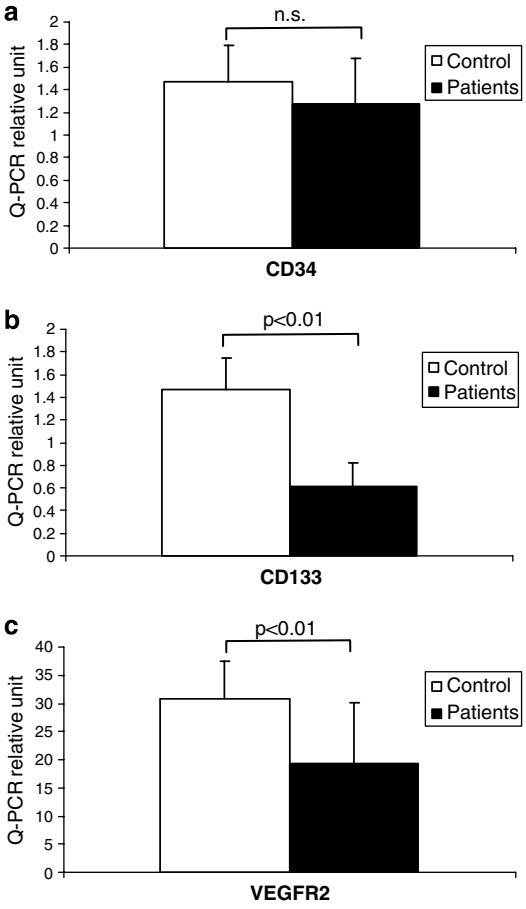

Figure 3 Relative quantification of CD34 (**a**), CD133 (**b**) and vascular endothelial growth factor receptor-2 (VEGFR2) (**c**) mRNAs by real-time quantitative reverse transcriptase (RT)-PCR in the peripheral blood of healthy controls and patients with depression. Results are expressed as means ± s.d. In (**a**), NS is non-significant.

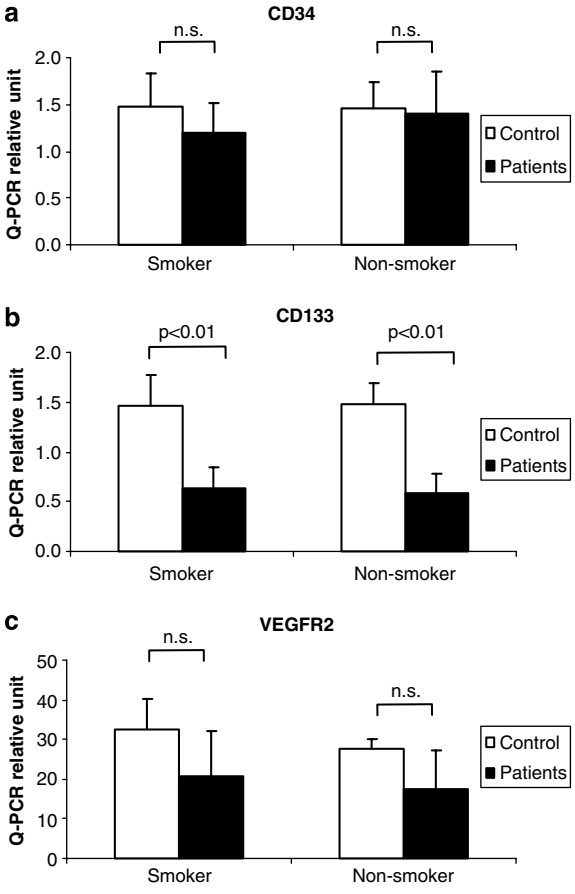

Figure 4 Relative quantification of CD34 (**a**), CD133 (**b**) and vascular endothelial growth factor receptor-2 (VEGFR2) (**c**) mRNAs by real-time quantitative reverse transcriptase (RT)-PCR in the peripheral blood of smoker and non-smoker subgroups of healthy controls and patients with depression. In (**a**, **c**), NS = non-significant.

the healthy controls versus that of the depressed patients. However, this tendency proved to be statistically significant only in the case of CD133 ($P < 0.01$ for both comparisons, Figure 4b).

Peripheral blood levels of VEGF and the proinflammatory cytokines TNF-α and CRP

Although patients with depression tended to have higher CRP levels than healthy controls, the difference between the two groups remained insignificant ($P = 0.29$, Table 1). Furthermore, we were unable to detect a significant relationship between CRP concentrations and circulating CD34+/VEGFR2+ or CD133+/VEGFR2+ EPC counts (*P*-values are 0.55 and 0.39, respectively, data not shown). However, TNF-α levels of patients were significantly elevated as compared with those of healthy controls ($P = 0.03$, Table 1) and, moreover, a statistically significant inverse correlation was observed between TNF concentrations and EPC numbers ($P < 0.05$, data not shown).

No significant difference was detected in the plasma levels of the key vasculogenic molecule, VEGF, between controls and patients ($P = 0.1$, Table 1). Furthermore, there was no statistically significant correlation between VEGF concentrations and circulating CD34+/VEGFR2+ or CD133+/VEGFR2+ EPC counts (both *P*-values are 0.08, data not shown).

Discussion

There is a growing body of evidence that blood vessel integrity not only depends on cells formerly residing within the vascular walls, but also is considerably controlled by bone marrow derived cells. Recent studies suggest that a subset of these cells, EPCs, may contribute to ongoing vascular repair by providing a circulating cell population that can home to the blood vessel walls and incorporate into the injured endothelial tube to replace dysfunctional endothelial cells.[14,36] Consequently, impairment of this EPC pool is considered to have negative effects on the cardiovascular system and patients with reduced numbers of EPCs are at increased risk for endothelial injury and for arteriosclerotic plaque development.[20–25]

A further established risk factor recognized today in the pathogenesis of arteriosclerosis and cardiovascular disorders is depression. However, although an association between mood disorders and susceptibility to cardiovascular events has been discovered by several researchers,[2,3,7–12,41–44] and altered circulating EPC levels have been reported in various conditions associated with vascular diseases,[20–25] to date no studies have attempted to evaluate the significance of EPCs in patients with major depression. Therefore, we investigated the significance of EPC numbers in our study sample by using flow cytometry and found a significant decrease in both mature (CD34+/VEGFR2+) and immature (CD133+/VEGFR2+) circulating EPC numbers in depressed patients versus healthy controls, and moreover, a statistically significant inverse relationship between EPC counts and the severity of depression independent of EPC phenotypes (as assessed by BDI scores[33]). Accordingly, although statistical significance was reached only for VEGFR2 and CD133 mRNA levels, there was a net trend toward a decrease in all the investigated EPC-specific mRNA (CD34, CD133 and VEGFR2) levels in patients with depression as compared with healthy controls. Interestingly, however, with the exception of CD133 mRNA levels, we failed to show a significant association between EPC-specific mRNA levels and EPC numbers. The reasons for this discrepancy between the results of flow cytometric analysis and PCR technique are not fully understood. However, it could be attributed to a complication that arises in using real-time quantitative RT-PCR to analyze mRNA levels in PB specimens. Although the PCR method offers the potential to rapidly and quantitatively analyze a number of gene products with limited material, the diversity of cellular populations present make it difficult to identify cell-specific gene expression patterns. In other words, possible reasons for the inconsistent results using two different techniques may include variations in EPC marker expression intensity at the stage of development in which they were studied, namely in the peripheral circulation following release from the bone marrow but prior to homing at the blood vessel site.

The finding that EPC levels are decreased in patients with depression may be secondary to a variety of mechanisms: depletion of the pool of EPCs in the bone marrow, reduced mobilization of the EPC population, or reduced survival and/or differentiation in the circulation. However, because VEGF is believed to be the most important among the molecules that participate in the regulation of EPCs,[14,18] one can assume that our observation on EPC numbers is the result of the reduced levels of this cytokine. Hence, we tested this hypothesis, but found normal VEGF levels in our patient population. Consequently, the possibility of insufficient bone marrow stimulation by VEGF in depression is not supported by the current results.

Recent experimental and clinical data also suggest that a variety of inflammatory mediators could be involved in the pathogenesis of low circulating EPC counts in depression.[42] Patients with depression who are otherwise medically healthy have been observed to have increased concentrations of proinflammatory cytokines and acute phase proteins. Elevated serum and/or plasma levels of CRP and/or interleukin-6 have been most frequently reported, although an increase in TNF-α plasma concentrations have also been described.[31] Combined with data demonstrating that CRP promotes apoptosis and attenuates the function and differentiation of EPCs[45] and TNF-α reduces the number of EPCs,[46] these findings suggested to us that CRP and/or TNF-α might promote EPC number reduction in depressed patients. Thus, we assayed the plasma levels of both cytokines and found that although CRP concentrations were, on average, higher in patients than in the control subjects, no statistically significant relationship existed between CRP concentrations and circulating progenitors. Nevertheless, the levels of TNF-α were significantly higher in the PB of patients when compared with the levels of healthy controls and, moreover, TNF-α levels inversely correlated with EPC counts. Therefore, the results of a recent study on rheumatoid arthritis reporting a significant increase in EPC levels after a single dose of infliximab, a monoclonal anti-TNF-α antibody,[47] might be interesting in this context. However, although the current data suggest a possible link between TNF-α and a decrease in circulating progenitors, chances are that as in other (for example, cardiovascular, malignant or inflammatory) disorders in which the interaction of multiple growth factors controls EPCs[14,18,36] the dynamic balance of several inflammatory and non-inflammatory cytokines is also likely to determine the number and function of these cells in depression. Therefore, additional studies are necessary to confirm the role of inflammation in modulating EPC numbers/function in depression.

The patient population analyzed in the current study had reduced numbers of EPCs but was free from confounding factors known to alter circulating EPC numbers, with the exception of cigarette smoking. Using flow cytometry, we found a significant decrease in CD34+/VEGFR2+ EPC counts among smokers, regardless of the population category (patients or controls). A similar tendency was observed among smokers in the case of CD133+/VEGFR2+ cells, although statistically significant reduction of EPCs with this phenotype was reached only in the control population. Nevertheless, these findings accord with the results of previous papers, in which smoking was demonstrated to decrease circulating EPC numbers.[25,40] Surprisingly, there were no significant correlations between the smoking status and the EPC-specific mRNA levels as evaluated by real-time quantitative RT-PCR. It is important to note, however, that because our patients and controls were carefully matched for smoking habits and because patients had always significantly lower EPC counts (both in the case of immature and mature EPCs, independent

of their smoking status), the significant decrease of EPC numbers and the tendency toward lower EPC-specific marker levels in the patient group are presumably attributable only to depression in the current study.

In addition to the relatively low number of participants, another limitation to this study was that most members of the patient group received some kind of psychotropic medicine while those in the control group were medication free (except for one person receiving diclofenac). However, the following observations, taken together, allow us to conclude that the observation of reduced EPC counts in our patients is significant, even in the presence of adequate pharmacological treatment. Firstly, none of the psychotropic drugs used in the current study has been reported to alter the number and/or function of EPCs. Secondly, the main categories of the psychotropic drugs used in the current study (benzodiazepines and selective serotonin reuptake inhibitor antidepressants) have been only very rarely observed to cause disturbances in the function of bone marrow (as a source of EPCs). Finally, and most importantly, because CRP and TNF-α levels are in an inverse relationship with the circulating numbers of EPCs[45,46] and because antidepressant agents were reported to decrease the elevated concentrations of these molecules,[48,49] one can assume that antidepressants rather increase than decrease the levels of EPCs.

In summary, EPCs are obviously involved in the regeneration of injured endothelium, and their number is thought to be a surrogate marker of vascular function.[36] The current study demonstrates for the first time that EPCs circulate in decreased numbers in the PB of patients with depression and, furthermore, opens a number of new perspectives in the pathogenesis of cardiovascular disorders in depression. However, although our results could be related to the EPC suppressive role of proinflammatory cytokines, further studies are needed to investigate the exact pathomolecular background of the connection between mood disorders and altered EPC counts. Moreover, it remains to be determined whether the EPC number abnormalities are state- or trait-dependent phenomena in affective disorders. Finally, our results also suggest that individuals should be screened for depression in future studies investigating EPC number and function in patient populations with cardiovascular risk factors (diabetes mellitus,[50] chronic renal failure,[51] smoking,[52] rheumatoid arthritis[53]) or with definitive cardiovascular diseases (myocardial infarction,[9] stroke[10]). It is especially crucial that the depression be considered an independent variable in such studies because depression is so highly comorbid with these cardiovascular conditions and diseases.

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