Congestive Heart Failure

CD34+ and Endothelial Progenitor Cells in Patients With Various Degrees of Congestive Heart Failure

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Background—Peripheral blood CD34+ cells and circulating endothelial progenitor cells (EPCs) increase in myocardial infarction and vascular injuries as a reflection of endothelial damage. Despite the occurrence of endothelial dysfunction in heart failure (HF), no data are available on EPC mobilization in this setting. We investigated the pattern of CD34+ cells and EPC mobilization during HF and their correlation with the severity and origin of the disease.

Methods and Results—Peripheral blood CD34+ cells (n=91) and EPCs (n=41), assessed both as CD34+ cells coexpressing AC133 and vascular endothelial growth factor (VEGF) receptor-2 and as endothelial colony-forming units, were studied in HF patients (mean age 67±11 years) and 45 gender- and age-matched controls. Tumor necrosis factor-α (TNF-α) and its receptors (sTNFR-1 and sTNFR-2), VEGF, stromal derived factor-1 (SDF-1), granulocyte-colony stimulating factor (G-CSF), and B-type natriuretic peptide were also measured. CD34+ cells, EPCs, TNF-α and receptors, VEGF, SDF-1, and B-type natriuretic peptide were increased in HF. CD34+ cells and EPCs were inversely related to functional class and to TNF-α, being decreased in New York Heart Association class IV compared with class I and II and controls. No role was found for the origin of the disease.

Conclusions—CD34+ cells and EPC mobilization occurs in HF and shows a biphasic response, with elevation and depression in the early and advanced phases, respectively. This could be related to the myelosuppressive role of TNF-α.

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Key Words: cells ▪ heart failure ▪ interleukins ▪ angiogenesis ▪ endothelium

Endothelial progenitor cells (EPCs) have been isolated from circulating CD34+ mononuclear cells and are thought to participate in vasculogenesis. When injected into animal models of ischemia or in humans, these cells are rapidly incorporated into sites of neovascularization and improve myocardial blood flow. Furthermore, EPCs may provide a circulating pool of cells that could form a cellular patch at the site of denuding injury or serve as a cellular reservoir to replace dysfunctional endothelium. EPCs and CD34+ cells increase in patients with endothelial damage, vascular trauma, and acute myocardial infarction, which reflects increased endothelial cell turnover.

Patients with heart failure (HF) show endothelial dysfunction. In HF, nitric oxide production is diminished, whereas rate of endothelial apoptosis is increased. Despite these observations, no data are available regarding the pattern of mobilization of EPCs and CD34+ cells during HF. We aimed to assess levels of circulating CD34+ cells and EPCs in patients with HF and correlate them with the origin and severity of the disease.

Methods

Patients

Ninety-one patients (74 men) aged 34 to 88 years (mean age 67±11 years) were consecutively enrolled. The control group comprised 45 healthy subjects who were matched as to gender and age (33 men; aged 65±6 years) with the patients. Ethics committee approval was obtained, as was informed consent from patients and controls. The Table shows the clinical details of the studied population. The diagnosis of HF was based on a history of congestive HF of at least 6 months’ duration (range 0.5 to 27 years), reduced exercise tolerance, and objective left ventricular functional impairment. The diagnosis of idiopathic dilated cardiomyopathy was based on accepted criteria. In 81 patients, a symptom-limited ergospirometric test was performed.

Patients were receiving standard medical treatment, which consisted of diuretics (86%), β-blockers (77%), ACE inhibitors (72%), aspirin (47%), warfarin (32%), and angiotensin II receptor blockers (22%). Statins had been discontinued in all patients at least 3 weeks before blood collection. The control group consisted of healthy subjects without any cardiovascular risk factor who were receiving no treatment.
Quantification of CD34+ Cells

Quantification of peripheral blood CD34+ cells was performed with double labeling, with FITC-anti-CD45 and phycoerythrin-anti-CD34 monoclonal antibodies (Becton Dickinson) on a FACSCalibur flow cytometer (Becton Dickinson) according to standardized procedures.10

EPC Enumeration

Enumeration of EPCs was performed as CD34+ cells coexpressing AC133 and vascular endothelial growth factor (VEGF) receptor-2 and as endothelial cultures (endothelial colony-forming units [e-CFUs]; Figure 1). It was performed on immunomagnetically purified peripheral blood CD34+ cells (Miltenyi Biotech) by triple labeling with peridinin chlorophyll protein–conjugated anti-CD34 (Becton Dickinson), phycoerythrin-conjugated anti-AC133 (Miltenyi Biotech), and unconjugated anti-VEGFR-2 (Santa Cruz Biotechnology), followed by FITC-conjugated swine anti-rabbit (Dako) as secondary reagent11 (Figure 1A). In a subset of patients and controls, the coexpression of CD45 was also confirmed.

Density gradient isolated peripheral blood mononuclear cells were resuspended in medium 199 (GIBCO BRL Life Technologies) supplemented with 20% fetal calf serum, penicillin, and streptomycin and plated on dishes coated with human fibronectin (Becton Dickinson). After 48 hours, nonadherent cells were collected and replated. Growth medium was changed every 3 days, and colonies were counted as described previously4 (Figure 1B through 1E). Confirmation of endothelial cell lineage was performed with antibodies against CD31 (Becton Dickinson), VEGFR-2, and factor VIII (Dako).

Cytokines and B-Type Natriuretic Peptide

Tumor necrosis factor-α (TNF-α) and its soluble receptors were assayed as described previously.12 VEGF, stromal derived factor-1 (SDF-1), granulocyte-colony stimulating factor, and B-type natriuretic peptide (BNP) were measured by ELISA (R&D) and by a solid-phase immunoradiometric assay (Shionoria), respectively.

Statistical Analysis

Data are shown as mean±SD. Comparisons between 2 or more groups were performed by Student t test and ANOVA, respectively; post hoc comparisons were by Turkey honest significance difference test. Correlations were tested by Pearson analysis. Probability was significant at a level of <0.05.

Results

The characteristics of the studied population are shown in the Table. Fifty-two patients (57%) had ischemic etiology, whereas 22 (24%) satisfied the criteria for idiopathic dilated cardiomyopathy. The remaining 17 patients had heart disease due to hypertension (n = 10), valvular disorders (n = 4), myocarditis (n = 2), and congenital disorder (n = 1).

CD34+, CD34+AC133 VEGFR2+ cells, and e-CFUs were higher in patients than in controls (Figure 2). TNF-α and its receptors sTNFR-1 and sTNFR-2, VEGF, SDF-1, and BNP were also elevated in patients (Table).

Figure 2A shows the distribution of CD34+ cells according to New York Heart Association class (P<0.0001). CD34+ cells were significantly decreased in class IV (1.7±1 cells/μL) and class III (2.4±0.9 cells/μL) compared with class II (3.3±1.5 cells/μL) and class I (4.9±2.0 cells/μL); they were increased in class I but decreased in class IV with respect to controls (P<0.0001 for both). CD34+AC133 VEGFR2+ cells (Figure 2B) and e-CFUs (Figure 2C) were elevated in class I (0.3±0.2 and 11±12 cells/μL, respectively) and class II (0.2±0.3 and 13±39 cells/μL, respectively) compared with class III (0.05±0.02 and 3.1±4.6 cells/μL, respectively) and class IV (0.03±0.03 and 2.5±1.5 cells/μL, respectively).

When patient population was divided according to BNP median levels (257 pg/mL), CD34+ and CD34+AC133 VEGFR2+ cells were both lower in those with high (2±1 and 0.04±0.02 cells/μL) than low (4±2 and 0.25±0.2 cells/μL; P<0.01 for both, respectively) BNP values. No effect of medical therapy (online
Correlations
CD34+ cells correlated to CD34+AC133+VEGFR2+ cells ($r=0.79; P<0.001$), peak oxygen consumption ($r=0.46; P<0.05$), New York Heart Association class ($r=-0.40; P<0.01$), and BNP ($r=-0.54; P<0.02$). CD34+ and CD34+AC133+VEGFR2+ cells correlated with TNF-$\alpha$ ($r=-0.67; P<0.01$ and $r=-0.59; P<0.05$, respectively), sTNFR-1 ($r=-0.77; P<0.01$ and $r=-0.63; P<0.05$, respectively), and sTNFR-2 ($r=-0.69; P<0.01$ and $r=-0.49; P<0.05$, respectively). SDF-1 was weakly correlated with e-CFUs ($r=0.39; P<0.03$). Renal function and cytokine levels did not correlate with CD34+ cells, CD34+AC133+VEGFR2+ cells, or e-CFUs.

Discussion
Our main finding is the peripheral recruitment of CD34+ cells and EPCs in early stages of HF, whereas in class IV, their peripheral mobilization was reduced not only with respect to class II and I but also with respect to controls. This yielded an

Figure 1. EPC enumeration. A, Flow cytometric characterization of peripheral blood EPCs. After gating on CD34+ cells that exhibited low side scatter (SSC) properties (left plot), EPCs were identified as CD34+ cells coexpressing AC133 and VEGFR-2 (right plot). B and C, Representative phase-contrast micrographs of EPC colonies, characterized by multiple thin, flat cells emanating from central cluster of rounded cells, shown at different degrees of magnification ($\times32$ in B and $\times200$ in C).

Figure 2. CD34+ cells and EPCs in study population. CD34+ cells (A), CD34+AC133+VEGFR2+ cells (B), and e-CFUs (C) in controls and patients, considered as whole population (All) or per New York Heart Association class. *$P<0.01$; †$P<0.001$; †† or ‡$P<0.005$; §$P<0.05$. Table available at http://www.circulationaha.org) or origin of HF was observed.
inverse correlation between CD34+ cells and the severity of the disease, whereas no role of etiology was observed.

Our previous work has shown that incubation of serum from HF patients induces endothelial cell apoptosis and endothelial nitric oxide synthase downregulation, which explains the severe endothelial dysfunction in this setting. Therefore, it is tempting to speculate that increased CD34+ and EPCs during HF may be a reflection of diffuse and severe endothelial damage.

The inverse correlation between CD34+ or CD34+/AC133+ VEGFR2+ cells and TNF-α and related soluble receptors led us to hypothesize a role of TNF-α in this biphasic response. During the early stages of the disease, when TNF-α is not yet significantly elevated, CD34+ and EPCs are increased as a reflection of a functional bone marrow response to diffuse and severe endothelial damage. In advanced disease phases, an additional and significant increase of TNF-α occurs that, by exerting a possible suppressive effect on hemopoiesis, finally counteracts and overwhims the triggers able to increase CD34+ and EPC mobilization during the early phases.

VEGF and SDF-1 were elevated in patients, and both tended to be further increased in advanced New York Heart Association classes. They might fail to effectively recruit EPCs during advanced phases of the disease, because of the contrasting effect of TNF-α on bone marrow cell mobilization. Recently, it was reported that granulocyte macrophage-CFUs are reduced and CD34+/CD133+ cells unchanged in the bone marrow from patients with ischemic cardiomyopathy, This suggests, together with the present data, that the response to HF might differ when peripheral mobilization is compared with resident bone marrow progenitor cells.

The present study was designed to investigate the pattern of EPC elevation in HF rather than their distinct phenotype. Further studies are needed to address this issue. Similarly, the causal contribution of EPC recruitment to HF remains to be determined.

In conclusion, more than 1 mechanism could be involved in the degree of CD34+ and EPC mobilization in HF, and our findings suggest that it could be stage dependent. An exhaustion of progenitor cells in the advanced phases of the disease could also contribute to the biphasic bone marrow pattern of response to HF.

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References
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