Vol. 50, No. 4, April 2004, pp 1296–1304
DOI 10.1002/art.20116

Circulating Endothelial Cells as a Marker of Ongoing Vascular Disease in Systemic Sclerosis

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Objective. **Circulating endothelial cells (CECs) have been described in different conditions involving vascular injury. Vascular abnormalities play a key role in the pathogenesis of systemic sclerosis (SSc). The aim of this study was to search for the presence of CECs in patients with SSc and to evaluate their clinical associations and possible pathogenic role.**

Methods. **The study cohort included 46 patients with SSc and 40 healthy controls. Five-parameter, 3-color flow cytometry was performed with a FACScan. CECs were defined as CD45 negative, CD34 positive, and P1H12 positive, and activated CECs were defined as CD45 negative and P1H12 positive, CD62 positive, or CD106 positive. Progenitors were identified as CD34 positive and CD133 positive.**

Results. **Total and activated CEC counts were significantly higher in SSc patients compared with healthy controls and were positively correlated with the disease activity score. With respect to visceral involvement, significant correlation was observed between the CEC number and the severity of pulmonary hypertension. High levels of endothelial progenitors were observed in patients with SSc, and the counts were higher in the early stages of disease.**

Conclusion. **The presence of CECs in patients with SSc may represent direct evidence of endothelial disease and may be a promising new clinical marker for active SSc. Notably, the association between CECs and pulmonary hypertension and impaired carbon monoxide diffusing capacity was evident in patients with limited cutaneous SSc only, suggesting an important role for CECs in this disease subset with prominent vascular changes. Detection of circulating endothelial progenitors may represent a response to vascular ischemia in early SSc, as an attempt at revascularization.**

Systemic sclerosis (SSc) is a connective tissue disease of unknown etiology and pathogenesis that is characterized by immunologic abnormalities, microangiopathy, and excessive deposition of collagen (1–3). Clinical and pathologic findings of vascular damage and endothelial cell activation strongly support the hypothesis of a unique vascular disease as an important and primary process in scleroderma (4,5). It is likely that prolonged endothelial cell perturbation and activation induced by ischemia and reperfusion lead to dysfunction and irreversible loss of integrity, with cell detachment and persistent tissue injury. Although an extensive body of literature supports the pathogenic importance of SSc vasculopathy (6), research in this field has been limited by the relative inaccessibility of vascular endothelium.

In certain disease conditions, circulating endothelial cells (CECs) detached from affected blood vessels provide useful material for studying vascular injury. In humans, CECs have been detected in diverse conditions having in common endothelial damage, such as coronary angioplasty, acute coronary syndrome, sickle cell anemia, thrombotic thrombocytopenic purpura, infection with *Rickettsia conorii* or cytomegalovirus, Behçet's disease, systemic lupus erythematosus (SLE), and small-vessel vasculitis (7–15). Moreover, it has been

Supported in part by a grant from the Associazione Italiana Leucemia, Sez Milano e Provincia.

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Submitted for publication April 19, 2003; accepted in revised form December 11, 2003.

suggested that in response to severe ischemia or cytokine stimuli, circulating endothelial cell progenitors (CEPs) increase and home into sites of angiogenesis and/or vascular damage, and consequently contribute to neovascularization and/or wound-healing processes (16–18).

The presence of CECs in these vascular disorders likely provides direct evidence of endothelial injury, even though it is unknown whether CECs correlate with the extent of endothelial lesions. Nevertheless, detection and analysis of CEC phenotype and/or CEPs may be an informative tool to study vascular dysfunction. Based on the hypothesis that severe vascular damage associated with SSc would result in endothelial shedding, we performed a cytometric evaluation of the number and surface phenotype of CECs in patients with SSc. We also determined whether the presence of CECs is associated with disease activity, clinical features, and/or plasma markers of endothelial activation.

A further goal of this study was to gain additional insight into the problem of vasculogenesis in SSc. Based on the hypothesis that the ischemic damage in SSc tissues might be associated with an attempt to recruit endothelial progenitors to sites of vascular damage, we assessed the presence of CEPs in peripheral blood obtained from patients with SSc and studied the correlation between CECs and CEPs and known angiogenic factors.

PATIENTS AND METHODS

Patients. Forty-six consecutive patients referred to our institution were included in the study. Informed consent was given by all patients. All patients studied fulfilled the American College of Rheumatology (formerly, the American Rheumatism Association) criteria for the classification of SSc (19). Patients were grouped according to the 2-cutaneous subset classification (1,20); namely, patients were classified as having limited cutaneous SSc (lcSSc; sclerosis of both distal extremities, not the elbow and knees, with or without sclerosis of the neck and face) or diffuse cutaneous SSc (dcSSc; sclerosis of both distal and proximal extremities, with or without truncal involvement). Patients with overlap symptoms to other connective diseases were excluded from the study.

The median disease duration was measured from the onset of the first signs and symptoms compatible with this disease (Raynaud's phenomenon, puffy hands, sclerodactyly with or without proximal scleroderma, dyspnea, and/or dysphagia).

All patients were treated with calcium channel blockers and/or angiotensin-converting enzyme inhibitors. Twenty-nine of 46 patients received therapy with intravenous prostanoid. The blood samples for CEC assay were always obtained before an iloprost infusion, and at least 1 month after the previous infusion. Because immunosuppressive drugs might theoretically interfere with CEC and CEP levels, patients receiving disease-modifying treatments (corticosteroids, cyclophosphamide, or methotrexate) were not included in the study.

Forty healthy subjects, matched with SSc patients for sex and age, were also included in this study as controls.

Clinical assessment. The clinical and laboratory data reported in this study are those obtained when the blood samples were collected. The patients included in the study had been evaluated for organ involvement at least 1 month before the blood sampling. Organ involvement was assessed according to previously described criteria (20). In particular, skin involvement was assessed by a modified Rodnan skin thickness score. The occurrence of pulmonary involvement was defined by pulmonary hypertension and/or bibasilar fibrosis on standard chest radiographs and/or restrictive lung disease. Pulmonary function was assessed by vital capacity, as measured by a dry spirometer, and by carbon monoxide diffusing capacity (DL_{CO}), as measured by the single-breath method, both of which are expressed as percentages. Pulmonary artery pressure was detected by color Doppler echocardiography. Joint/tendon involvement was defined by the detection of symmetric synovitis, flexion contractures, and tendon friction rubs (21). Muscle involvement was evaluated as isolated muscle weakness or weakness associated with elevated levels of serum creatine kinase with or without electromyographic or histologic changes of inflammatory myopathy. The degree of inflammatory activity was determined by the erythrocyte sedimentation rate (ESR) and the C-reactive protein (CRP) level.

For all patients with SSc, disease activity was assessed using the activity indices described by the European Scleroderma Study Group (22,23). According to these criteria, disease was considered active if the sum of the scores for detected items was ≥ 3. Disease stages were defined as suggested by Medsger and Steen (24): early lcSSc (disease duration <5 years), intermediate/late lcSSc (disease duration ≥ 5 years), early dcSSc (disease duration <3 years), and intermediate/late dcSSc (disease duration ≥ 3 years).

Detection of CECs by flow cytometry analysis. Two hundred microliters of peripheral blood in sodium heparin was labeled with 10 μl of a panel of fluorescein isothiocyanate (FITC)–, R-phycoerythrin (R-PE)–, or peridin chlorophyll protein–conjugated antibodies anti-CD45, anti-CD34, anti-CD106, anti-CD133, anti-CD62, and anti-P1H12, for 20 minutes at room temperature. After conjugation, red blood cells were lysed by incubating in FACS lysing solution (Becton Dickinson, San Jose, CA) for 15 minutes at room temperature. White blood cell pellets were then washed twice in FACSFlow solution (Becton Dickinson).

Appropriate analysis gates were used to enumerate total and activated CECs and CEPs. CECs were defined as negative for CD45 and positive for CD34 and P1H12 (9,25). Activated CECs were defined as negative for CD45, positive for P1H12, and positive for CD62 or CD106 (9,25). CD133 and CD34 surface expression characterized CEPs (17). Five-parameter, 3-color flow cytometry was performed with a FACScan flow cytometer with a 15-mW argon laser (excitation at 488 mm) (Becton Dickinson). The sensitivity of fluorescence detectors was set and monitored using CaliBRITE beads (Becton Dickinson) according to the manufacturer's recommendations. Cells stained with isotypic controls for IgG1–FITC or R-PE were used as negative controls (26). At least 100,000 (typically 300,000) cells per sample were acquired; analyses were considered informative when adequate numbers of events (>100)

Table 1. Assessment of disease activity in patients with systemic sclerosis*

Patient no.	Modified Rodnan score >14	Scleroderma	Δ skin	Digital necrosis	Δ vascular	Arthritis	DLco <80%	Δ Heart/ lung	ESR >30 mm/hour	Hypocomple-mentemia	Total maximum DA score
1	1	1	2	0.5	0.5	0	0	0	0	0	5
2	0	0	0	0	0	0	0.5	2	0	0	2.5
3	0	0	0	0.5	0.5	0.5	0.5	0	0	1	3
4	0	0	0	0.5	0.5	0.5	0	0	0	0	1.5
5	0	0	0	0.5	0.5	0	0.5	0	0	0	1.5
6	0	0	0	0	0	0	0.5	2	0	0	2.5
7	1	0	0	0.5	0	0	0.5	0	0	0	2
8	1	1	2	0	0.5	0.5	0.5	2	1.5	1	10
9	1	0	0	0.5	0.5	0	0.5	0	0	0	2.5
10	0	0	2	0	0.5	0	0.5	2	1.5	0	6.5
11	0	0	0	0.5	0	0	0	2	0	0	2.5
12	0	0	0	0	0	0.5	0.5	0	1.5	1	3.5
13	0	0	0	0	0	0.5	0.5	0	0	0	1
14	0	0	0	0	0	0	0.5	2	0	0	2.5
15	0	1	2	0	0.5	0	0.5	2	0	0	6
16	1	0	0	0.5	0.5	0.5	0.5	2	1.5	1	7.5
17	0	0	0	0.5	0.5	0.5	0	0	0	0	1.5
18	0	0	0	0	0.5	0	0.5	0	0	0	1
19	0	1	0	0	0.5	0	0.5	0	0	0	2
20	1	0	0	0	0	0	0	0	0	0	1
21	1	1	2	0	0.5	0.5	0.5	2	1.5	0	9
22	1	1	2	0	0	0	0.5	2	1.5	0	8
23	1	1	2	0	0.5	0	0.5	0	0	0	5
24	1	1	2	0.5	0	0	0.5	2	1.5	0	8.5
25	1	1	2	0.5	0.5	0	0.5	0	1.5	0	7
26	1	0	0	0	0.5	0.5	0	0	0	0	2
27	0	0	0	0	0.5	0.5	0.5	2	1.5	1	6
28	0	1	2	0.5	0.5	0	0.5	0	0	0	4.5
29	0	1	0	0	0.5	0.5	0	0	0	0	2
30	1	0	2	0	0.5	0	0.5	0	0	0	4
31	1	1	2	0.5	0	0	0.5	0	0	0	5
32	1	1	2	0.5	0.5	0	0.5	2	1.5	0	9
33	0	0	0	0	0	0.5	0.5	0	0	0	1
34	1	0	0	0	0	0	0	0	0	0	1
35	0	1	0	0	0.5	0	0.5	2	1.5	0	5.5
36	1	1	0	0	0	0	0.5	0	1.5	0	4
37	0	1	2	0	0	0	0.5	0	1.5	0	5
38	1	1	0	0	0	0	0.5	2	1.5	0	6
39	1	0	2	0	0.5	0	0.5	0	0	0	4
40	0	1	0	0.5	0.5	0	0.5	0	0	0	2.5
41	1	0	2	0	0.5	0.5	0	0	1.5	0	5.5
42	1	0	2	0	0.5	0.5	0.5	2	1.5	0	8
43	1	0	0	0	0	0	0.5	2	1.5	0	5
44	0	0	0	0	0	0	0.5	0	0	0	0.5
45	0	0	0	0	0.5	0	0.5	0	0	0	1
46	1	0	0	0	0.5	0	0.5	2	0	0	4

* See ref. 23 for definitions of the variables used to calculate the disease activity (DA) score. DLco = carbon monoxide diffusion in the lung; ESR = erythrocyte sedimentation rate.

were collected in the CEC enumeration gates (25). Data were analyzed with CellQuest software (Becton Dickinson).

Detection of vascular endothelial growth factor (VEGF) and soluble E-selectin. Plasma concentrations of soluble E-selectin and VEGF were assessed by commercial enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN) and calculated using a standard curve generated with specific standards, according to the manufacturer's recommendations.

Statistical analysis. Comparisons of CEC levels between patients with SSc and healthy controls were performed by the Mann-Whitney rank sum test. P values less than 0.05 were considered significant. In addition, correlations between CEC number and different SSc disease parameters were assessed by Spearman's rank correlation test.

RESULTS

Demographic and clinical features of patients. The present study included 45 women and 1 man, with a median age of 56 years (range 24–72 years). Twenty-two

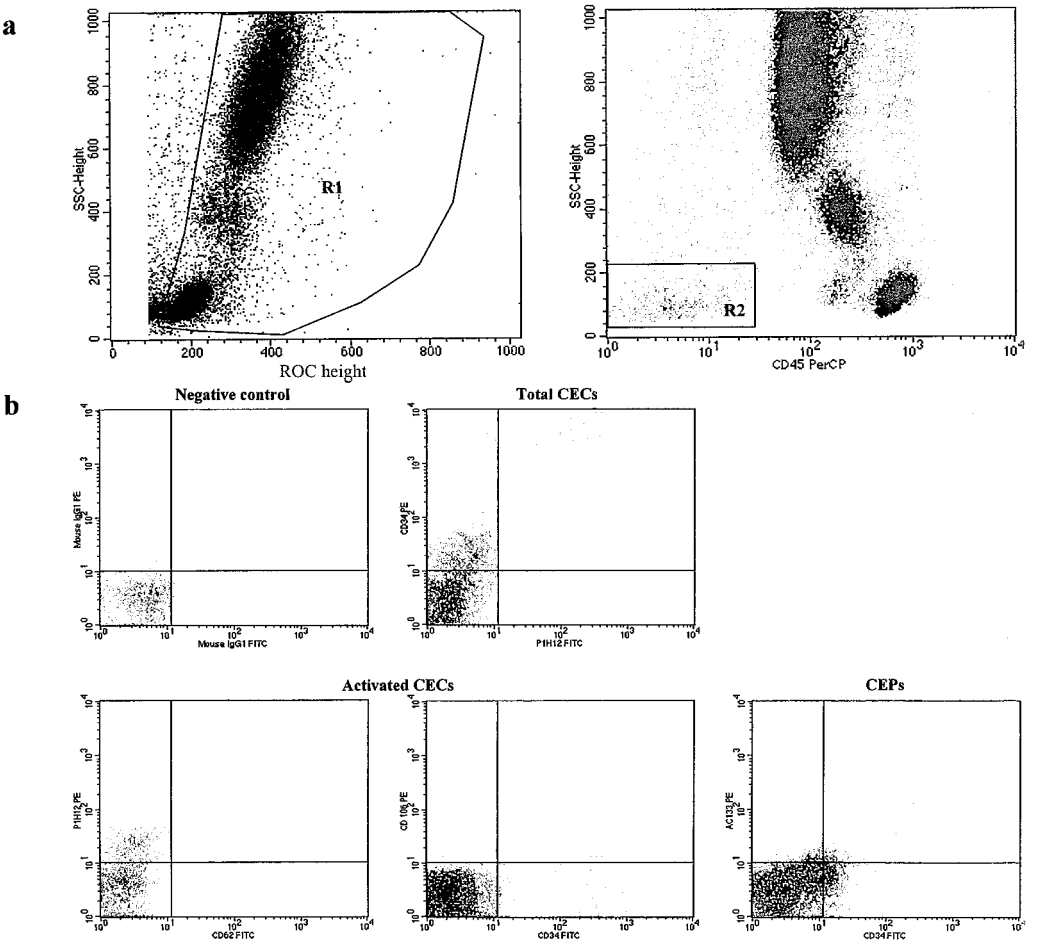

Figure 1. Flow cytometry evaluation of circulating endothelial cells (CECs) and circulating endothelial cell progenitors (CEPs). **a,** Representative panel showing the analysis gate used to exclude platelets and debris (left), and the gate used to exclude CD45-positive hematopoietic cells (right). **b,** Representative panel showing the negative control, total and activated CECs, and CEPs in systemic sclerosis (SSc). ROC = receiver operating curve; PerCP = peridin chlorophyll protein; PE = phycoerythrin; FITC = fluorescein isothiocyanate.

patients were classified as having lcSSc, and 24 patients were classified as having dcSSc. The median disease duration was 5.5 years (range 1–23 years). According to Medsger and Steen's criteria (24), 9 (40.9%) of 22 patients with lcSSc and 5 (20.8%) of 24 patients with dcSSc had early disease.

All patients reported the occurrence of Raynaud's phenomenon after exposure to low temperatures. Twenty-three patients (50%) had a modified Rodnan skin thickness score of >14. Cutaneous ulcers were observed in 15 (32.6%) of 46 patients. With respect to pulmonary involvement, 11 (23.9%) of 46 patients with SSc had pulmonary fibrosis with pulmonary hypertension, and 14 (30.4%) of the 46 patients had primary pulmonary hypertension. Evaluation of disease activity by the European Scleroderma Study Group criteria (22,23) indicated that 27 (58.7%) of 46 patients had active disease (Table 1).

Quantitative studies of CECs. We identified CECs as negative for hematopoietic marker CD45 and positive for endothelial markers and CD34 (Figure 1). The mean number of CECs in patients with SSc (310 cells/ml; 95% confidence interval [95% CI] 243–375) was significantly higher than that in healthy controls (77 cells/ml; 95% CI 69–110) ($P < 0.001$) (Table 2). When analyzed according to disease subset, both patients with limited SSc and patients with diffuse SSc showed significantly increased levels of CECs in comparison with healthy controls ($P < 0.05$ and $P < 0.001$, respectively). Compared with patients with limited SSc, those with

Table 2. Levels of total and activated CECs and CEPs in SSc patients and healthy controls*

	SSc	Diffuse SSc	Limited SSc	Controls
Total CECs	310 (243–375)	376 (292–461)	236 (136–337)	77 (69–110)
Activated CECs				
CD62+	56 (38.4–73.3)	62 (37.3–86.8)	49 (24.3–81.7)	11 (0–27.9)
CD106+	65 (48.3–81.5)	84 (59.4–108.3)	44 (22.6–75.6)	22 (4.2–39.8)
CEPs	122 (78–166.9)	119 (68–170)	127 (49–203.8)	28 (0–56)

* Values are the mean (95% confidence interval). For total circulating endothelial cells (CECs), $P < 0.001$ for systemic sclerosis (SSc) and diffuse SSc versus controls, and $P < 0.05$ for limited SSc versus controls. For all activated CECs and circulating endothelial cell progenitors (CEPs), $P < 0.05$ for SSc, diffuse SSc, and limited SSc versus controls.

diffuse SSc had a higher mean number of CECs, but the difference did not reach statistical significance (376 cells/ml and 236 cells/ml, respectively).

CECs showed a strong correlation with disease activity ($r_s = 0.728$, $P = 0.001$), and the number of CECs was significantly higher in patients with active SSc than in patients with clinically inactive disease ($P = 0.001$). No correlation was found between CEC levels and digital necrosis, skin parameters (i.e., total skin thickness score, scleroderma), or joint and muscle involvement. Regarding pulmonary involvement, CECs showed a significant correlation with pulmonary pressure values in the group of all patients with SSc ($r_s = 0.379$, $P < 0.05$) (data not shown) and in the subset of patients with limited SSc ($r_s = 0.515$, $P < 0.05$) (Figure 2A). Moreover, a significant negative correlation between CECs and DLCO was observed only in patients with limited SSc ($r_s = -0.416$, $P < 0.05$) (Figure 2B), not in all patients with SSc or in the subset with diffuse SSc. No significant differences in CEC levels were observed between patients treated with prostanoid and untreated patients.

Activated phenotype of CECs. To assess whether CECs are in an activated state, we analyzed them for dual expression of P1H12 and molecules that are expressed on the endothelial surface upon activation. The number of CECs with surface expression of E-selectin or vascular cell adhesion molecule 1 (VCAM-1) (CD62 and CD106, respectively) was markedly increased in patients with SSc compared with that in healthy controls ($P = 0.01$ and $P = 0.001$, respectively) (Table 2) and represented ~20% of the total CECs. The number and phenotype of activated CECs were not different between patients with diffuse SSc and those with limited SSc. The level of CECs positive for these activation markers was higher in patients with active disease (namely, patients with a disease activity score of >3) than in patients in a steady state and showed a significant correlation with the activity score ($r_s = 0.357$, $P = 0.05$) (data not shown). No correlation between activated CEC counts and digital necrosis, skin involvement, or pulmonary parameters was found.

The mean (±SD) plasma concentration of

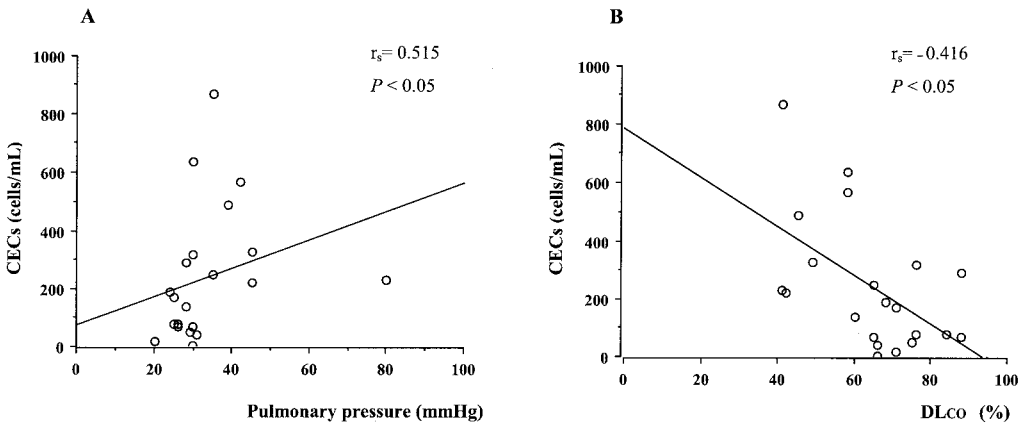

Figure 2. Correlations of circulating endothelial cell (CEC) counts with pulmonary artery pressure values (**A**) and carbon monoxide diffusion in the lung (DLCO) (**B**) in the subset of patients with limited cutaneous systemic sclerosis.

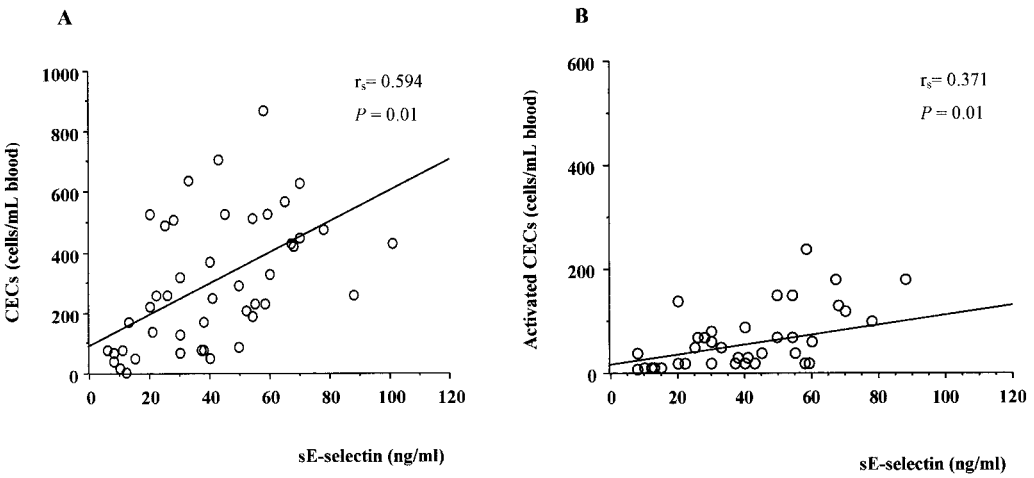

Figure 3. Correlations of plasma levels of soluble E-selectin (sE-selectin) with concentrations of total (**A**) and activated (**B**) circulating endothelial cells (CECs) in patients with systemic sclerosis.

E-selectin was 42.30 ± 23.7 ng/ml in patients with SSc and 8.8 ± 8.7 in healthy subjects ($P < 0.0001$). As shown in Figure 3, the plasma E-selectin level in patients with SSc strongly correlated with the number of total and activated CECs ($r_s = 0.594$, $P = 0.01$, and $r_s = 0.371$, $P = 0.01$, respectively). Moreover, E-selectin levels showed a strong correlation with the disease activity score ($r_s = 0.604$, $P < 0.05$) (data not shown).

Quantitative studies of CEPs. According to Rafii (17), endothelial cell progenitors were identified by surface expression of CD34 and CD133, a novel stem cell marker that is expressed on CEPs but not on mature endothelial cells. As shown in Table 2, CEP concentrations were significantly higher in patients with SSc than in healthy controls ($P < 0.05$). No significant correlation with clinical or pathologic parameters (namely, digital necrosis, skin and visceral involvement, and disease activity score) was found. Notably, when patients with SSc were stratified according to duration of disease, those with recent-onset disease (<5 years for lcSSc and <3 years for dcSSc) showed an increased number of CEPs in comparison with patients with chronic disease ($P < 0.01$ and $P < 0.05$, respectively) (data not shown).

VEGF is a major angiogenic peptide with specific mitogenic activity on vascular endothelial cells; it is normally secreted by cells under hypoxic conditions and is involved in several steps of angiogenesis (27,28). Plasma concentrations of VEGF were increased in patients with SSc compared with healthy controls (median 507 pg/ml [range 74–1,250 pg/ml] versus median 97 pg/ml [range not detectable–248 pg/ml]; $P < 0.001$) (data not shown), but no positive correlation was found between circulating mature or progenitor endothelial cells and VEGF.

DISCUSSION

The aim of our study was to investigate the presence of CECs in patients with SSc and to evaluate whether the number of CECs could have some relationship to the different subsets and stages of the disease. The results of the study show that 1) patients with SSc have high levels of total and activated CECs, 2) the presence and number of CECs are related to high levels of disease activity and, with regard to organ involvement, to the presence of pulmonary hypertension, 3) the number of both total and activated CECs correlates with plasma concentration of soluble E-selectin, and 4) endothelial cell progenitors were also detectable in peripheral blood from patients with SSc, and their number was significantly higher in patients with early-stage disease.

To our knowledge, the finding of increased levels of CECs in patients with SSc has not been previously reported. However, the amount of CECs detectable in peripheral blood has been recently proposed as a reliable marker of endothelial damage in different vascular diseases, such as acute coronary syndrome (also after coronary angioplasty), sickle cell anemia, thrombotic thrombocytopenic purpura, rickettsial and cytomegalovirus infections, Behçet's disease, SLE, and small-vessel vasculitides (7–15). Thus, the detection of CECs in patients with SSc is certainly not surprising, because results of several histologic studies of dermal microvas-

culature in this disease, which demonstrated disruption of the normal architecture of the endothelium with loss of intercellular junctions and progressive death of endothelial cells (3–6), have suggested that endothelium may play a key role in the pathologic process of SSc. Whatever the underlying mechanisms of microvascular damage in SSc are, it is likely that prolonged and repeated endothelial derangement may lead to irreversible loss of integrity, with detachment, apoptosis, and necrosis of endothelial cells.

The number of CECs detected in patients with SSc is not different in the subsets of patients with limited or diffuse variants of the disorder. This is not surprising, because for both lcSSc and dcSSc several studies have demonstrated widespread vascular damage in lesional as well as prelesional tissues (5,29,30). Moreover, the amount of CECs found in SSc is comparable with that reported in patients with different diseases characterized by very extensive vascular injury, such as sickle cell anemia, rickettsial and cytomegalovirus infections, and antineutrophil cytoplasmic antibody–associated vasculitides (9,11,12,15). The fact that SSc (among the vasculopathies described in other connective tissue diseases) is characterized by peculiar and particularly aggressive endothelial damage is further confirmed by our preliminary results obtained in patients with SLE. By using the same method for detection of CECs, we observed that in 15 patients with active SLE the mean number of CECs was higher than that in healthy subjects, but was only moderately elevated when compared with that in patients with SSc (mean ± SEM 310 ± 32 cells/ml and 99 ± 4.2 cells/ml, respectively; $P < 0.05$) (Del Papa N: unpublished observations). This finding strongly supports the conclusion that, besides the common feature of a widespread vascular injury that is believed to be effective in all connective tissue diseases, completely different pathogenetic mechanisms may underlie any single disorder.

Markers of endothelial cell activation and damage, such as von Willebrand's factor, endothelin 1, soluble VCAM, and E-selectin, have been considered good candidates to assess either the activity or the severity of the disease (31). Our data, showing that the amount of CECs closely correlated with disease activity, strongly suggest that these cells could be an additional marker of active disease in patients with SSc. The clinical relevance of our results is further reinforced by the fact that we first used a validated method for the assessment of the disease activity (23). In contrast, previous studies assumed that a short duration of disease together with its rapid progression, and modification of some acute-phase reactants or parameters (e.g., the ESR and the CRP level) could quite well define those patients with active disease (32–35).

The fact that the number of CECs correlated with the disease activity scores as a whole, and not with any single clinical feature usually thought to be indicative of active disease, is not contradictory. The weight of any single item included in a complex activity scale, such as that used in the present study, can be, in fact, largely variable, and the absolute prevalence of a single clinical parameter, even if considered to be a strong marker of active disease in a single patient, can be quite low in the whole population of patients.

As far as organ involvement is concerned, we found a close correlation between increased levels of CECs and the presence of pulmonary hypertension in the whole population of patients with SSc, particularly in those with lcSSc. In the subset of patients with lcSSc, an impairment of gas exchange was also associated with the presence of high levels of CECs. These results may be explained by the different mechanisms predominantly involved in determining pulmonary hypertension in patients with lcSSc, with respect to those effective in patients with dcSSc. Several studies have consistently shown that damaged and dysfunctional endothelium plays a central and critical role in the initiation and progression of pulmonary hypertension in SSc (36,37). The consequences of persistent endothelial dysfunction include vasospasm, vascular remodeling, and thrombosis, which are responsible for both increased pulmonary vascular resistance and a mismatched ventilation–perfusion ratio (38,39). These mechanisms, which can be ascribed to a pure pulmonary endothelial disease, are predominantly active in patients with lcSSc. Such patients have a higher risk for the development of progressive pulmonary hypertension in the absence of any interstitial fibrotic change. In contrast, in patients with dcSSc, development of pulmonary hypertension usually occurs late in the course of the disease and is commonly related to pulmonary fibrosis, which has been demonstrated often to be the final result of evolved pulmonary alveolitis (36,37).

The finding that a relevant number of CECs have a proadhesive phenotype is consistent with previous data indicating that endothelial cells are present in their activated form in the small vessels of patients with SSc. This has been suggested by several studies that demonstrated an up-regulation of E-selectin and intercellular adhesion molecule (ICAM) in the skin of patients with SSc (29,40–43), and increased plasma concentrations of soluble forms of endothelial cell surface adhesion mol-

ecules, including E-selectin, ICAM-1, and VCAM-1 (44,45). E-selectin is the adhesion molecule most restricted to endothelial cells, and, therefore, its increased levels in plasma and overexpression in tissues of patients with SSc may be strictly related to endothelial activation. In our study, this issue is confirmed by the finding that patients with SSc also had significantly raised plasma E-selectin concentrations, and that CEC counts strongly correlated with plasma E-selectin levels. Based on these observations, activated CEC counts may offer a useful approach for the evaluation of endothelial activation in vivo.

The detection of high numbers of CEPs in patients with SSc, particularly in those in the early stages of disease, suggests an attempt at revascularization and healing of ischemic tissues. It is thought that the loss of functional and structural integrity of endothelium in SSc might be an important stimulus for angiogenic processes and may well explain the increase in the level of circulating VEGF found in our study, as in previous studies (46,47). These findings are consistent with recent reports showing how the endothelial cell precursors may migrate to the sites of active neovascularization in ischemic tissues and may differentiate in situ into mature endothelial cells (18). Further studies, however, are needed to completely elucidate the real significance and role of CEPs in the pathogenetic mechanisms of vascular damage and repair in SSc.

In conclusion, our data provide evidence for the presence of a relevant number of circulating mature endothelial cells in patients with SSc, as a probable result of shedding from affected walls of the blood vessels. The correlation between the number of CECs and disease activity strongly suggests that the CEC count may be a promising marker for vascular damage and disease progression, particularly with regard to development of pulmonary hypertension in patients with the limited cutaneous variant of SSc. Furthermore, the possibility of identifying patients with active vascular damage could be useful both for selecting patients suitable for a more aggressive therapeutic approach and for monitoring the efficacy of such a therapeutic intervention. This study may provide a basis on which to test these hypotheses in larger, longitudinal studies.

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