Low levels of endothelial progenitor cells correlate with disease duration and activity in patients with Behçet's disease

G.P. Fadini[1], S. Tognon[2], L. Rodriguez[2], E. Boscaro[3], I. Baesso[3], A. Avogaro[1], G. Semenzato[3], C. Agostini[3], R. Marcolongo[3]

[1]Department of Clinical and Experimental Medicine, Metabolic Division, [2]Department of Neurological Sciences, Ophthalologic Clinic, [3]Department of Clinical and Experimental Medicine, Clinical Immunology, University of Padova, Medical School, Padova, Italy.

Abstract

Objective

We tested whether Behçet's disease (BD) is characterized by alterations of circulating endothelial progenitor cells (EPCs),which are involved in vascular homeostasis and repair.

Methods

We enrolled 30 BD patients and 27 matched healthy controls. EPCs were defined and measured by flow cytometry according to the expression of CD34, CD133 and KDR.

Results

We show that BD patients had significantly lower levels of CD34+KDR+ and CD34+CD133+KDR+ EPCs than controls. We found significant negative correlations between EPC phenotypes and BD duration, while there were positive correlations between CD34+KDR+ EPCs and both BD activity scores and C-reactive protein. The lower EPC levels with increasing disease duration was shown in univariate analysis and in multivariable analysis adjusted for possible confounders.

Conclusions

This is the first report that BD is associated with progressive EPC decline. Reduction of EPCs may represent a mechanism of induction and/or progression of vascular injury in these patients.

Key words

Endothelium, Behçet's disease, stem cells, vasculitis.

Gian Paolo Fadini, MD
Sofia Tognon, MD
Laura Rodriguez, MD
Elisa Boscaro, BSc
Ilenia Baesso, BSc
Angelo Avogaro, MD
Gianpietro Semenzato, MD
Carlo Agostini, MD
Renzo Marcolongo, MD

Please address correspondence to:
Carlo Agostini, MD,
Department of Clinical and Experimental Medicine, Clinical Immunology,
Via Giustiniani 2,
35100 Padova, Italy.
E-mail: carlo.agostini@unipd.it

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Introduction

Behçet's disease (BD) is a relapsing multisystemic inflammatory disorder with unknown etiology, but likely determined by the simultaneous contribution of genetic, environmental and immunologic factors (1). BD has many features in common with systemic vasculitides and it is generally agreed that initiation of vascular damage in these disorders is mediated by exaggerated immune responses against self-antigens located in the vessel wall. However, an eventual autoantigen has not been identified, such that the triggers of these reactions are unknown, and the mechanisms driving progression of vascular lesions are incompletely understood. The first vascular barrier against blood-borne reactions is represented by the endothelial layer, which plays a major role in maintaining vascular health, as it regulates vasorelaxation, adhesiveness and fibrinolysis (2). For instance, endothelial damage opens the way to vascular inflammation, which subsequently leads to atherosclerosis in subjects with predisposing risk factors, such as hypercholesterolemia (3). In analogy, a primary endothelial damage may represent a trigger for deranged immune responses against self antigens in subjects with predisposing genetic background and autoreactive T lymphocytes, thus leading to vasculitis.

Integrity of the endothelial layer relies on the slow local endothelial cell turnover and on the critical contribution of circulating cells, namely endothelial progenitor cells (EPCs) (4). EPCs derive from the bone marrow and are mobilized in the peripheral circulation in response to many stimuli including ischemia and vascular damage(5, 6). Once in the bloodstream, EPCs constitute a pool of cells that can actively repair endothelial layer discontinuities by forming a cellular patch at sites of denudation (7). Moreover, these cells are directly involved in physiologic and pathologic angiogenesis, as they integrate into the nascent vessel endothelium (8). Thanks to these properties, EPCs play major roles in cardiovascular homeostasis, and it has been shown in humans that reduction of circulating EPCs associates with endothelial dysfunction and vascular damage (9-11).

In this study, we quantified circulating EPCs in BD patients and control healthy subjects in order to test the hypothesis that vascular inflammation associated with BD is characterized by defective endothelial regeneration.

Material and methods

Patients

A total of 30 patients with BD were recruited at the Immunology or Ophthalmic clinics of the University of Padova (Italy). The inclusion criterion was BD with ocular or systemic involvement, independently of disease stage and treatment. Exclusion criteria were: any concomitant acute disease or infection, recent (within 3 months) surgery or trauma, overt cardiovascular disease (ruled out with minimal criteria including history and physical examination), diabetes, hypertension, familiar hypercholesterolemia, pregnancy and lactation. Diagnosis of BD was confirmed according to international criteria (12). In parallel, 27 matched control subjects were selected from a local community of office employees who underwent a health screening. As a disease control group, we also recruited 7 patients with active pulmonary sarcoidosis, in whom diagnosis was confirmed by histological criteria (13): 3 of them also had sarcoidosis related ocular manifestations. We collected the following data for all subjects: age, sex, smoking habit (of one or more cigarettes per day), family history for cardiovascular disease in first degree relatives, BMI (body mass index calculated as body weight in kg divided by squared height in m), systolic and diastolic blood pressure. Blood samples were collected for the determination of plasma glucose, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides concentrations. C-reactive protein was also measured as an index of inflammation. In BD patients we also collected the following data: disease duration (defined as time elapsed since diagnosis), presence/absence of the HLA-B51 susceptibility locus, current use of corticosteroids or immunosuppressive drugs. BD severity was estimated using

scores constructed on the basis of the diagnostic criteria proposed by the International Scientific Committee on Behçet's disease (14). We obtained a lifetime score of ocular and extraocular manifestations, as well as a score of ocular and extraocular manifestations at time of blood sampling for this study. Scores ranged from 0 to 20 as previously described for ocular (15) and global activity (16).

Flow cytometry

Circulating progenitor cell level was determined in fasting blood samples using direct three-color flow cytometry, as previously described in detail (17). Since there is no agreement on the antigenic definition of EPCs, we quantified different putative progenitor cell populations on the basis of the expression of three surface antigens: the stemness markers CD34 and CD133 and the endothelial marker KDR (*i.e.* type 2 VEGF receptor). Briefly, after red cell lysis, whole blood cells were stained with a FITC-conjugated anti-CD34 (Bekton Dickinson), a APC-conjugated anti-CD133 (clone AC133, Miltenyi Biotec) and a PE-conjugated anti-KDR (R&D Systems). We first gated on CD34 or CD133 positive cells, which could be identified as a distinct cell population with high expression of the above

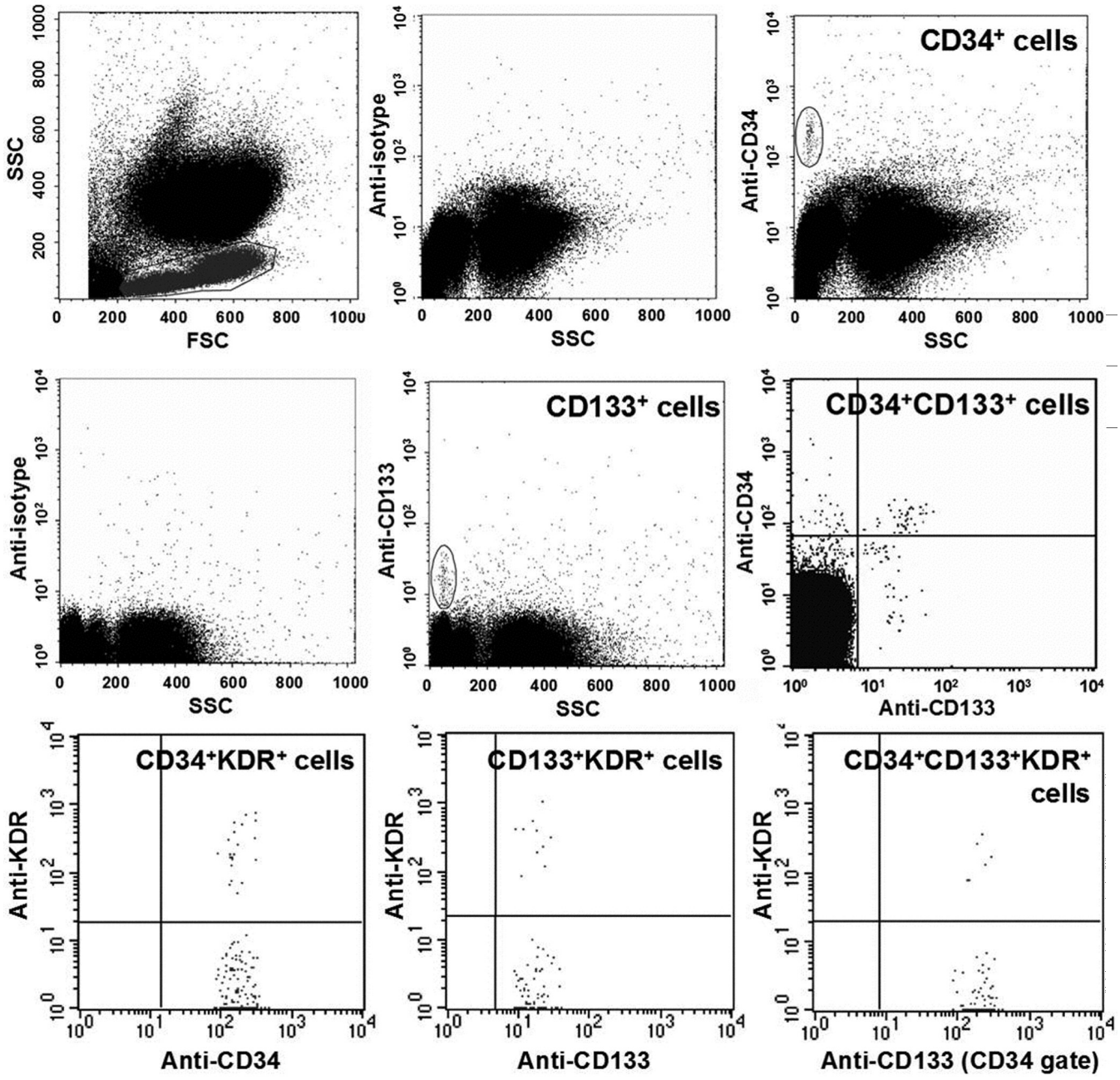

Fig. 1. The gating strategy used to enumerate circulating progenitor cells. CD34+ and CD133+ cells were identified as cells with low side scatted (SSC) and high fluorescent intensity of the specific monoclonal antibody. At the intersection of CD34+ and CD133+ gates, double positive CD34+CD133+ cells were identified. These cell populations were then assayed for the expression of KDR in the mononuclear cell fraction, to identify CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+ cells.

mentioned antigens and low side scatter, and then assayed these cells for the expression of KDR in the mononuclear cell fraction. CD34/CD133 double positive cells were identified at the intersection between the CD34 and CD133 gates, and these cells were further assayed for KDR expression (Fig. 1). CD34+, CD133+ and CD34+CD133+ cell populations were considered generic circulating progenitor cells (CPCs), as being positive for stemness antigens. CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+ cells were considered EPCs, as being also positive for the endothelial marker. For all analyses, 1×10^5 events were collected and scored using a FACS Calibur analyser (Bekton Dickinson). Data were processed using the Macintosh CELLQuest software program (Becton Dickinson), and cell count was expressed per one million cytometric events.

Statistical analysis

Data are presented as mean ± standard error for continuous variables or median (range) for categorical data. Normal distribution was checked using the Kolmogorov-Smirnov test. Comparison between two groups was performed using Student's *t*-test for continuous normally distributed data and Mann-Whitney test for non-normal variables. The χ^2 test was used for dichotomous variables. Bonferroni correction was applied to control for α-error inflation due to multiple testing. Linear correlations between continuous variables were assayed with the Pearson's coefficient. To identify variables independently associated with EPC level, we ran a stepwise multiple linear regression. SPSS 13.0 was used and statistical significance was accepted at $p\leq0.05$.

Results

Patients' characteristics

Characteristics of control and BD subjects are reported in Table I. BD patients were relatively young (mean age 36.2±1.7) and sexes were almost equally distributed (17 men and 13 women). The analysis of cardiovascular risk parameters confirm that these subjects were free from overt alterations in blood pressure, glucose and lipid metabolism.

Table I. Characteristics of the study subjects.

Characteristic	Control subjects (n=27)	BD patients (n=30)	Sarcoidosis (n=7)
Age, years	36.3 ± 1.0	36.2 ± 1.7	32.9 ± 2.2
Sex, M/F	12/15	17/13	3/4
Smoking habit, n (%)	11 (40.7)	16 (53.3)	2 (29)
Family history for CVD, n (%)	13 (48.1)	12 (40.0)	1 (14)
Body mass index, kg/m^2	23.2 ± 0.7	23.9 ± 0.5	24.0 ± 1.1
Systolic blood pressure, mmHg	120.2 ± 2.1	123.3 ± 1.7	121.9 ± 2.1
Diastolic blood pressure, mmHg	82.3 ± 1.2	78.8 ± 1.2	80.8 ± 1.1
Plasma glucose, mg/dl	86.2 ± 2.3	84.6 ± 1.8	86.3 ± 2.0
Total cholesterol, mg/dl	192.4 ± 7.1	192.1 ± 9.1	190.9 ± 11.1
HDL-cholesterol, mg/dl	53.1 ± 2.4	56.7 ± 2.7	54.2 ± 4.0
LDL-cholesterol, mg/dl	117.6 ± 7.8	114.6 ± 7.3	112.5 ± 6.7
Triglycerides, mg/dl	87.1 ± 10.5	104.3 ± 11.3	102.9 ± 18.3
C-reactive protein, mg/l	0.84 ± 0.17	3.04 ± 0.78*	2.78 ± 1.2*

*significantly different as compared to control subjects after α-correction.

Control subjects were fully matched for age, sex and cardiovascular parameters. The only significant difference between the two groups was the higher mean CRP concentration in BD patients versus controls, which is consistent with the chronic inflammatory state that accompanies BD. Patients with sarcoidosis were also matched with control and BD patients (Table I).

BD patients had a median disease duration of 6.0 years (interquartile range 3.0-11.8). They were characterized by a predominant ocular involvement, since all of them had active (29/30) or had had previous (30/30) ocular disease. Sixty percent of BD patients were positive for HLA-B51. A total of 11 patients were on chronic corticosteroid therapy (mean dose 0.24 mg/kg), while 10 were on immunesuppressive drugs (6 were taking both drug classes). Immunosuppressive drugs were distributed as follows: 6 with cyclosporine A, 2 with infliximab, 1 with azathyoprine and 1 with methotrexate. Disease manifestations and activity were distributed as reported in Table II.

Progenitor cell levels are reduced in BD patients

Six phenotypes of progenitor cells were determined in peripheral blood of BD patients and healthy matched controls. Three of these (CPCs) are to be considered cell populations that include the progeny of different cell lineages, including endothelial. The other 3 phenotypes (EPCs) are a subsample of CPC and display signs of endothelial priming as evidenced by the expression of the endothelial antigen KDR. We show a strong significant reduction of the levels of CD34+KDR+ (37.2±5.5 versus 71.5±10.4; p=0.004) and CD34+CD133+KDR+ (2.7±0.8 versus 8.7±1.4; p<0.001) EPCs phenotypes in BD patients as compared to control subjects. A trend for reduction of CD133+, CD34+CD133+ and CD133+KDR+ cells was also present, but was not statistically significant (Fig. 2). These results allowed us to concentrate only on

Table II. BD manifestations. Scores are presented as median (range).

Criteria	Score
Lifetime extraocular manifestations (0-5)	
Oral aphthosis	2 (0-4)
Genital aphthosis	0 (0-3)
Skin lesions	1 (0-4)
Joint pain	1 (0-4)
Vascular lesions	0 (0-3)
Urological symptoms	0 (0-2)
Central nervous system symptoms	0 (0-3)
Gastroenteral lesions	0 (0-4)
Astenia / cephalgia / fever	1 (0-2)
Total	7.5 (3-18)
Current extraocular manifestations (0-5)	1 (0-4)
Lifetime ocular manifestations (0-5)	3 (0-5)
Current ocular manifestations (0-5)	0 (0-3)

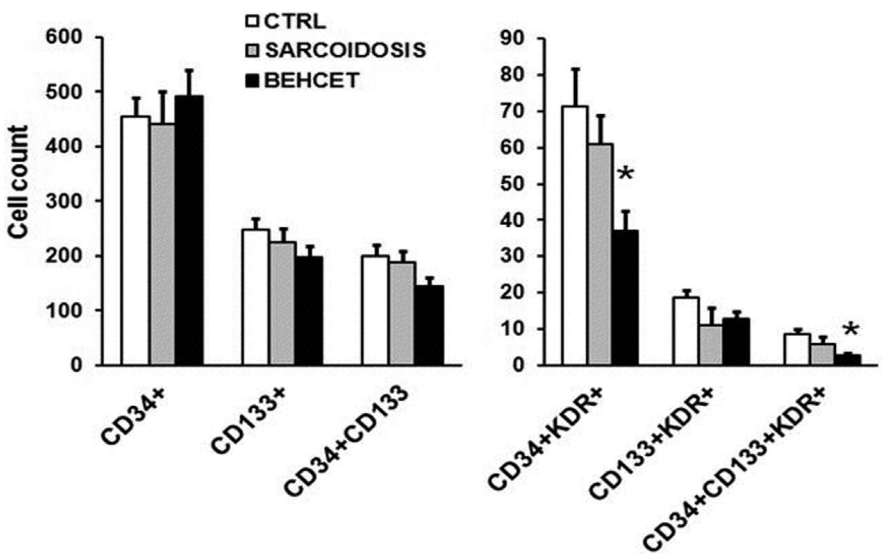

Fig. 2. Circulating progenitor cells (CPC, CD34+, CD133+, CD34+CD133+) and endothelial progenitor cells (EPCs, CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+) in control subjects and BD patients. * $p<0.05$ BD versus controls.

EPC phenotypes in subsequent analyses. Patients with sarcoidosis showed slight and not significant reduction in EPC phenotypes (Fig. 2).

EPC levels correlate with BD duration and activity

In BD patients, we found no significant correlation between EPC levels and cardiovascular risk parameters, such as plasma glucose, lipids, blood pressure, and smoking. Concerning BD characteristics, we found a significant negative correlation between all the 3 EPC phenotypes and BD duration (CD34+KDR+: r=-0.54; $p<0.001$; CD133+KDR+: r=-0.42; p=0.01; CD34+CD133+KDR+: r=-0.37; p=0.03), which remained significant after adjustment for age (p=0.005; 0.04; 0.04 respectively). There was also a significant positive

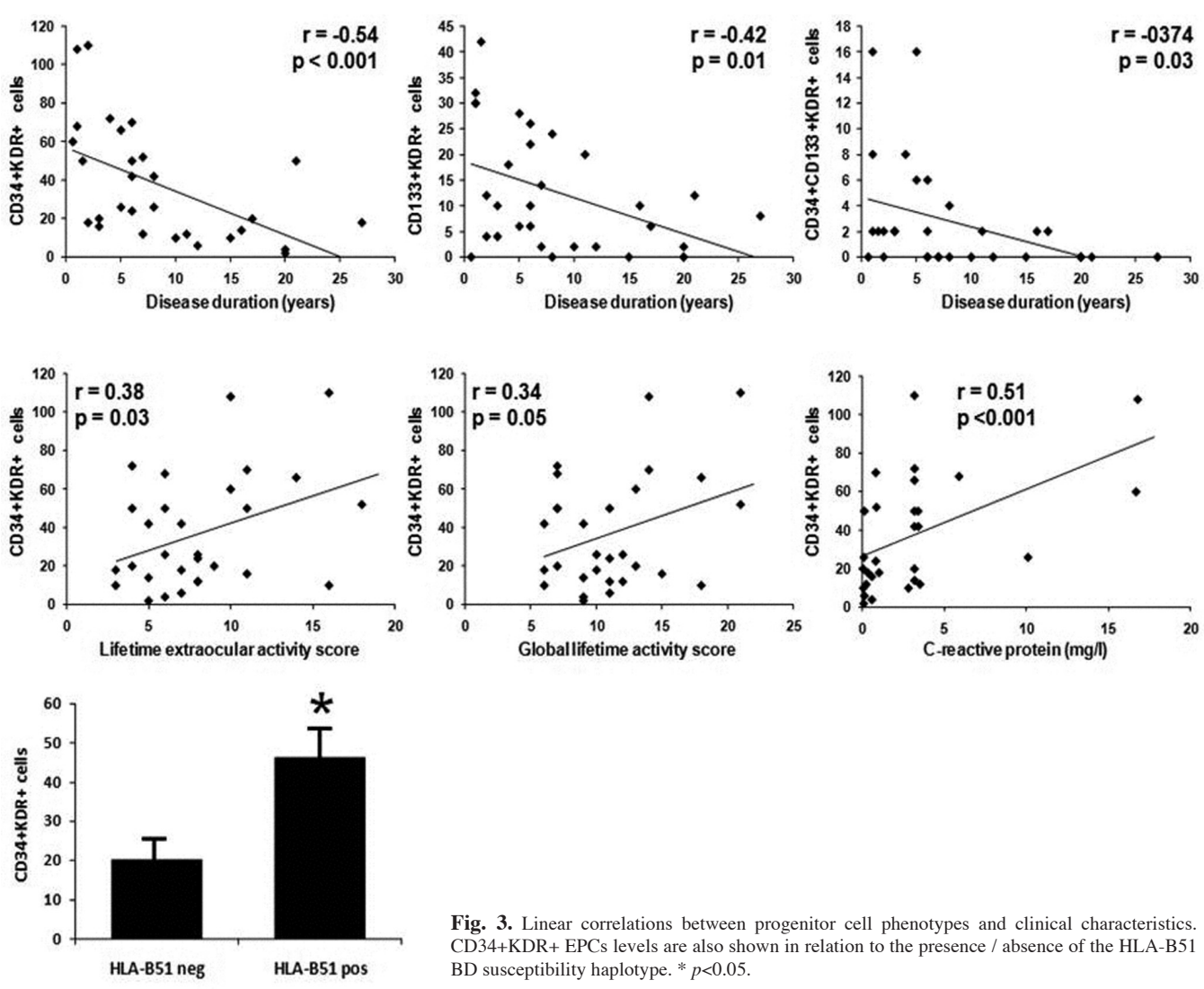

Fig. 3. Linear correlations between progenitor cell phenotypes and clinical characteristics. CD34+KDR+ EPCs levels are also shown in relation to the presence / absence of the HLA-B51 BD susceptibility haplotype. * $p<0.05$.

correlation between CD34+KDR+ EPCs and lifetime extraocular (r=0.38; p=0.03) and global BD activity score (r=0.34; p=0.05), but this was not true for the other 2 EPC phenotypes. There were no correlations between progenitor cell levels and ocular activity scores or activity scores at time of the study. Moreover, we found no significant differences in cell counts according to BD activity and type of organ damage (*e.g.* venous versus arterial involvement). The levels of CD34+KDR+ EPCs were also directly correlated with C-reactive protein concentrations (r=0.51; p<0.001). Finally, carriers of the HLA-B51 susceptibility locus had significantly higher CD34+KDR+ EPCs (46.3±7.2 in B51-positives versus 20.3±6.3 in B51-negatives; p=0.016). There were no associations between any of the progenitor cell phenotypes and treatment regimens. Given the amount of significant associations found with CD34+KDR+ cells (Fig. 3), which is consistent with the notion that this is the best EPC phenotype (18), we then focused on this specific cell population for further analyses.

Disease duration is an independent determinant of EPC level in BD

To identify variables independently associated with CD34+KDR+ cell count, we used a multiple regression analysis. The stepwise approach was chosen because of the high number of explanatory variables to be entered in the model. We found that EPC levels were associated with disease duration independently of other factors, such as activity score, HLA-B51 and therapy (Table III).

Table III. Results of a stepwise multivariable linear regression analysis. Dependent variable was CD34+KDR+ cell count. The stepwise approach reveals that disease duration was the only covariable independently associated with EPC level.

	Standardized b coefficient	*p*-value
Variables in the model		
Disease duration	-0.539	0.003
Excluded variables		
Age	-0.030	0.878
Sex	0.043	0.827
Systolic blood pressure	0.105	0.594
Diastolic blood pressure	-0.088	0.657
Body mass index	0.369	0.063
Plasma glucose	0.175	0.373
Total cholesterol	0.096	0.628
HDL cholesterol	-0.286	0.141
Triglycerides	0.085	0.669
C-reactive protein	0.085	0.669
Smoking habit	-0.244	0.211
Family history	0.019	0.925
HLA-B51 positive	0.221	0.258
Corticosteroid therapy	-0.079	0.691
Immunesuppression	-0.154	0.435
Lifetime global activity score	0.245	0.208

Discussion

In this study, we demonstrate for the first time that patients with BD have a reduced level of circulating EPCs in comparison with fully matched control subjects. In light of the prominent role of EPCs in vascular biology, these new findings may provide a novel perspective on the mechanisms initiating or promoting vascular damage in BD, and in systemic vasculitis in general. Being that no significant reduction of EPCs were found in patients with active sarcoidosis, we suggest that this might be a more specific feature of vascular inflammation.

Systemic vasculitides are characterized by inflammatory infiltrates inside the vessel wall, leading to a variety of clinical manifestations depending upon site, diameter, and type (arterial versus venous) of the involved blood vessels (19). BD typically targets both small and large arteries as well as veins. In addition to systemic non-specific symptoms (such as fever, myalgia and joint pain), progression of vascular disease can lead to arterial obstruction, aneurismatic remodeling, and venous thrombosis as well. Such an inflammatory state might derive from an autoimmune reaction against still undefined autoantigens in the vessel wall. Unmasking of self antigens that are normally not exposed to the immune system, and/or appearance of autoreactive T cells may open the way to vascular inflammation. While micro-organism infection and molecular mimicking may be the first trigger, this hypothesis has not been definitely confirmed (1).

Experimental and human studies have repeatedly shown that reduction/dysfunction of EPCs is associated with endothelial dysfunction, endothelial damage and a reduced ability to repair the vessel wall after injury (3, 9, 11). Therefore, we suggest that defects in EPCs may play a double role in the early and late phases of the natural history of BD vasculitis. First, a primary EPC defect may favor endothelial damage, subendothelial exposition, subtle inflammation and eventual unmasking of self-antigens. This would represent a trigger for autoimmune reactions in predisposed individuals. Second, in the later phases of vascular damage, a low level of EPCs would hamper endothelial repair and reconstitution of a healthy intimal layer, thus amplifying damage progression. This model is also supported by the observation of reduced EPC levels in patients with ANCA-associated vasculitis (20, 21). Previous studies of endothelial biology in vasculitis led to the demonstration of higher levels of circulating endothelial cells (CECs) in these patients (22, 23). Contrary to EPCs, CECs are mature cells shed off the vessel wall after injury (24, 25). Therefore, while EPCs represent the endogenous endothelial regenerative potential, CECs are indicators of endothelial damage.

In addition to the reduced EPCs in BD than in control subjects, the most consistent result of the present study in that EPC decline is strongly related to BD duration. Similar results have been reported for many other clinical conditions, such as diabetes and lung

disease (26, 27). Aging is one of the major determinant of EPC levels (17, 28), but here we show that the negative correlation between BD duration and EPC count was independent of age and other covariates. This finding may be interpreted as a sort of exhaustion of the EPC pool due to disease chronicity. Indeed, conditions in which the vasculature is stressed, such as burns, tissue ischemia and vascular trauma, are known to induce a potent mobilization of EPC from the bone marrow into the peripheral circulation, with the aim of re-establishing vascular integrity (healing) and blood supply (angiogenesis) (5, 6, 8). Actually, we show that some patients with a more active disease had higher EPC levels, suggesting that EPC mobilization might occur during bursts of vascular inflammation. This is also indicated by the observation that patients with inflammatory recrudescence (higher CRP) showed higher EPC levels, and is in compliance with previous studies on the effects of acute inflammation on EPCs (29). While acute inflammation increases EPCs, over the long term, a chronic inflammatory state might be accompanied by a progressive EPC reduction, as demonstrated by previous clinical and experimental studies (30, 31). Similar results were found in other clinical conditions characterized by phases of acuteness and progression towards chronic states. For example, while unstable angina pectoris is characterized by increased EPC levels (5), chronic ischemic heart disease evolves with progressively reduced EPCs (32). That disease chronicity is the major negative modulator of EPC level is confirmed by the multivariable analysis showing BD duration as the only independent variable associated with EPC count. However, an alternative hypothesis is that decreased EPCs in long-lasting disease may reflect a lower level of bone marrow stimulation due to a decrease in vascular damage and ischemia, or as a result of effective treatment.

One unexpected finding is the higher mean EPC level in carriers versus non-carriers of the HLA-B51 susceptibility haplotype. While there are some data in support of a genetic determination of EPC levels (33), this observation has not a clear explanation and deserves further investigation. However, this association may be due to chance or confounded by immunosuppressive regimens: indeed the results of the multiple regression indicates that the association of EPCs with disease duration is stronger than those with activity scores, CRP, and HLA.

Keeping in mind that EPCs are also mediators of neoangiogenesis, the progressive reduction of EPC level over time may have other implications in the light of the role played by angiogenesis in vasculitides (34). For instance, ocular angiogenesis, which has been experimentally shown to involve EPCs, usually takes place only in the early stages of BD, when EPCs levels are higher, than in the later stages. Thus, inflammatory bursts in early productive BD may favor angiogenesis through the mobilization of EPCs, conditioning disease progression and evolution. On the contrary, low EPC levels in long-lasting BD would significantly hamper compensatory angiogenesis in the case arterial occlusion leading to ischemic syndromes. Finally, the biphasic EPC regulation in early and late BD may reflect alternatively protection or susceptibility to atherosclerosis, which is not a prominent feature of BD (35).

We were not able to demonstrate a correlation between EPCs and ocular disease activity. However, this should not be interpreted as an evidence against a role for EPC in ocular BD manifestations, because most of the patients included in this study had a predominant ocular BD.

Limitations

Although this study establishes a strong association between BD and reduced EPCs, its cross-sectional nature does not allow conclusions on the cause-effect relationships between EPCs and BD development or progression. In addition, most BD patients included in this study were on treatment or had received immunosuppression with different regimens. Since some immunosuppressive drugs are known to affect EPC biology (36-38). this may represent a confounder in the relationship between EPC levels and disease activity. We previously showed that fertile women have higher levels of circulating CD34+KDR+ EPCs than age-matched men, and that gender is one major determinant of EPCs in healthy subjects (39). While the presence of fertile women in the present study may confound results, it is noteworthy that gender is no more an independent predictor of EPC levels. This may suggest that the presence of BD eliminates the advantage conferred by the female sex on vascular health. Finally, due to the predominance of ocular involvement, BD patient population included in this study might not really represent the full disease spectrum.

We acknowledge the methodological uncertainties inherent to the quantification of circulating EPCs (18, 40). Here, we used a widely standardized flow cytometry method, which allowed the antigenic definition of EPCs by staining for three different surface markers. It is noteworthy that, among the three putative EPC phenotypes, the CD34+KDR+ provided the highest number of significant correlations with clinical variables. This is consistent with an extensive review of the literature, yielding that this phenotype is the best compromise in terms of measure validity and biological meaning, when EPCs are meant as disease biomarkers (18). The less significant results obtained with the other two EPC phenotypes partly depend on the higher variability of their measure. Finally, isolation and culture of EPCs from BD patients would strengthen our results, but there is considerable uncertainties regarding the biological significance of *in-vitro* expanded EPCs (40).

Conclusions

Patients with BD have a lower level of circulating EPCs than matched controls. Although some patients with more active disease may have a transient EPC mobilization, disease chronicity leads to a progressive EPC depletion over the long term. Speculatively, reduced EPCs, through a deranged vascular homeostasis, may be both a trigger of the autoreactive immune response, and an amplification factor of tissue damage.

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References

1. KRAUSE I, WEINBERGER A: Behçet's disease. *Curr Opin Rheumatol* 2008; 20: 82-7.
2. LANDMESSER U, DREXLER H: The clinical significance of endothelial dysfunction. *Curr Opin Cardiol* 2005; 20: 547-51.
3. FADINI GP, AGOSTINI C, SARTORE S, AVOGARO A: Endothelial progenitor cells in the natural history of atherosclerosis. *Atherosclerosis* 2007; 194: 46-54.
4. URBICH C, DIMMELER S: Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res* 2004; 95: 343-53.
5. GEORGE J, GOLDSTEIN E, ABASHIDZE S *et al*.: Circulating endothelial progenitor cells in patients with unstable angina: association with systemic inflammation. *Eur Heart J* 2004; 25: 1003-8.
6. GILL M, DIAS S, HATTORI K *et al*.: Vascular trauma induces rapid but transient mobilization of VEGFR21AC1331 endothelial precursor cells. *Circ Res* 2001; 88: 167-74.
7. WERNER N, PRILLER J, LAUFS *et al*.: Bone marrow-derived progenitor cells modulate vascular reendothelialization and neointimal formation: effect of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibition. *Arterioscler Thromb Vasc Biol* 2002; 22: 1567-72.
8. TAKAHASHI T, KALKA C, MASUDA H *et al*.: Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat Med* 1999; 5: 434-8.
9. HILL JM, ZALOS G, HALCOX JP *et al*.: Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003; 348: 593-600.
10. FADINI GP, SARTORE S, ALBIERO M *et al*.: Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arterioscler Thromb Vasc Biol* 2006; 26: 2140-6.
11. FADINI GP, CORACINA A, BAESSO I *et al*.: Peripheral blood CD34+KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population. *Stroke* 2006; 37: 2277-82.
12. INTERNATIONAL STUDY GROUP FOR BEHÇET'S DISEASE: Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-80.
13. JUDSON MA: The diagnosis of sarcoidosis. *Clin Chest Med* 2008; 29: 415-27.
14. CHANG HK, LEE SS, BAI HJ *et al*.: Validation of the classification criteria commonly used in Korea and a modified set of preliminary criteria for Behçet's disease: a multi-center study. *Clin Exp Rheumatol* 2004; 22: S21-26.
15. TOGNON S, GRAZIANI G, MARCOLONGO R: Anti-TNF-alpha therapy in seven patients with Behçet's uveitis: advantages and controversial aspects. *Ann N Y Acad Sci* 2007; 1110: 474-84.
16. INTERNATIONAL SOCIETY FOR BEHÇET'S DISEASE: http://www.Behçet's.ws/pdf/BehçetsDiseaseActivityForm.pdf
17. FADINI GP, DE KREUTZENBERG SV, CORACINA A *et al*.: Circulating CD34+ cells, metabolic syndrome, and cardiovascular risk. *Eur Heart J* 2006; 27: 2247-55.
18. FADINI GP, BAESSO I, ALBIERO M, SARTORE S, AGOSTINI C, AVOGARO A: Technical notes on endothelial progenitor cells: ways to escape from the knowledge plateau. *Atherosclerosis* 2008; 197: 496-503.
19. KRISHNAMURTHY A, NAGUWA SM, CHEEMA GS, GERSHWIN ME: Vasculitis: contemporary perspectives. *Compr Ther* 2006; 32: 118-26.
20. ZAVADA J, KIDERYOVA L, PYTLIK R, VANKOVA Z, TESAR V: Circulating endothelial progenitor cells in patients with ANCA-associated vasculitis. *Kidney Blood Press Res* 2008; 31: 247-54.
21. BROGAN PA: What's new in the aetiopathogenesis of vasculitis? *Pediatr Nephrol* 2007; 22: 1083-94.
22. NAKATANI K, TAKESHITA S, TSUJIMOTO H, KAWAMURA Y, TOKUTOMI T, SEKINE I: Circulating endothelial cells in Kawasaki disease. *Clin Exp Immunol* 2003; 131: 536-40.
23. WOYWODT A, STREIBER F, DE GROOT K *et al*.: Circulating endothelial cells as markers for ANCA-associated small-vessel vasculitis. *Lancet* 2003; 361: 206-10.
24. SHANTSILA E, WATSON T, LIP GY: Endothelial progenitor cells in cardiovascular disorders. *J Am Coll Cardiol* 2007; 49: 741-52.
25. BOOS CJ, LIP GY, BLANN AD: Circulating endothelial cells in cardiovascular disease. *J Am Coll Cardiol* 2006; 48: 1538-47.
26. TEPPER OM, GALIANO RD, CAPLA JM *et al*.: Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. *Circulation* 2002; 106: 2781-6.
27. FADINI GP, AVOGARO A, AGOSTINI C: Pathophysiology of circulating progenitor cells in pulmonary disease and parallels with cardiovascular disease. *Am J Respir Cell Mol Biol* 2006; 35: 403-4.
28. HEISS C, KEYMEL S, NIESLER U, ZIEMANN J, KELM M, KALKA C: Impaired progenitor cell activity in age-related endothelial dysfunction. *J Am Coll Cardiol* 2005; 45: 1441-8.
29. RAFAT N, HANUSCH C, BRINKKOETTER PT *et al*.: Increased circulating endothelial progenitor cells in septic patients: correlation with survival. *Crit Care Med 2007;* 35: 1677-84.
30. CESARI F, CAPORALE R, MARCUCCI R *et al*.: NT-proBNP and the anti-inflammatory cytokines are correlated with endothelial progenitor cells' response to cardiac surgery. *Atherosclerosis* 2008; 199: 138-46.
31. FUJII H, LI SH, SZMITKO PE, FEDAK PW, VERMA S: C-reactive protein alters antioxidant defenses and promotes apoptosis in endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2006; 26: 2476-82.
32. HEESCHEN C, LEHMANN R, HONOLD J *et al*.: Profoundly reduced neovascularization capacity of bone marrow mononuclear cells derived from patients with chronic ischemic heart disease. *Circulation* 2004; 109: 1615-22.
33. WHITTAKER A, MOORE JS, VASA-NICOTERA M, STEVENS S, SAMANI NJ: Evidence for genetic regulation of endothelial progenitor cells and their role as biological markers of atherosclerotic susceptibility. *Eur Heart J* 2008; 29: 332-8.
34. MARUOTTI N, CANTATORE FP, NICO B, VACCA A, RIBATTI D: Angiogenesis in vasculitides. *Clin Exp Rheumatol* 2008; 26: 476-83.
35. SEYAHI E, YAZICI H: Atherosclerosis in Behçet's syndrome. *Clin Exp Rheumatol* 2007; 25 (Suppl. 45): S1-5.
36. WANG CH, CHERNG WJ, YANG NI *et al*.: Cyclosporine increases ischemia-induced endothelial progenitor cell mobilization through manipulation of the CD26 system. *Am J Physiol Regul Integr Comp Physiol* 2008; 294: R811-818.
37. DAVIES WR, WANG S, OI K *et al*.: Cyclosporine decreases vascular progenitor cell numbers after cardiac transplantation and attenuates progenitor cell growth *in vitro*. *J Heart Lung Transplant* 2005; 24: 1868-77.
38. GRISAR J, ALETAHA D, STEINER CW *et al*.: Endothelial progenitor cells in active rheumatoid arthritis: effects of tumour necrosis factor and glucocorticoid therapy. *Ann Rheum Dis* 2007; 66: 1284-8.
39. FADINI GP, DE KREUTZENBERG S, ALBIERO M *et al*.: Gender differences in endothelial progenitor cells and cardiovascular risk profile: the role of female estrogens. *Arterioscler Thromb Vasc Biol* 2008; 28: 997-1004.
40. INGRAM DA, CAPLICE NM, YODER MC: Unresolved questions, changing definitions, and novel paradigms for defining endothelial progenitor cells. *Blood* 2005; 106: 1525-31.