Systemic lupus erythematosus patients exhibit functional deficiencies of endothelial progenitor cells

J. Grisar¹, C. W. Steiner¹, M. Bonelli¹, T. Karonitsch¹, I. Schwarzinger², G. Weigel³, G. Steiner¹,⁴ and J. S. Smolen¹,⁴

Objective. SLE is characterized by an increased cardiovascular risk. Since endothelial progenitor cells (EPCs) have been described to serve as a biomarker for the CV risk and are known to be depleted in various diseases, we were interested if SLE would also be associated with altered peripheral EPC levels or functional abnormalities of these cells.

Methods. EPCs were quantified in 31 female SLE patients with different disease activity and in age-matched healthy controls (HCs) by FACS analysis and by colony forming unit (CFU) assay. Furthermore, EPC adhesion and migration capacity were tested.

Results. EPC levels were similar in HC and SLE when assessed by FACS (0.045 ± 0.006% vs 0.036 ± 0.007% within the lymphocyte gate) and by the CFU assay (18 ± 3 vs 15 ± 2 colonies/well). No correlation with disease activity could be observed, but SLE patients treated with chloroquine exhibited significantly decreased EPC levels (0.058 ± 0.005% without vs 0.024 ± 0.008% with chloroquine, P < 0.05). Addition of chloroquine to in vitro cultures also led to a decreased colony formation in SLE and in HC. When testing the adhesion and migration capacity of EPC on human umbilical vein endothelial cells (HUVEC), cells from SLE patients had reduced adhesion (19.2 ± 3.5% vs 36.6 ± 5.2% EPC/high power field, P < 0.02) and migratory activity (56 ± 6 cells/random microscopic field in SLE vs 121 ± 28 in controls, P < 0.02).

Conclusion. The data reveal that EPCs are significantly affected in SLE. While circulating EPC levels are in the range of HC, they exhibit functional deficiencies that may lead to impaired tissue availability.

Key words: Systemic lupus erythematosus, Endothelial progenitor cells, Cardiovascular risk, Chloroquine, Transmigration.

Introduction

SLE is associated with an increased risk of cardiovascular events leading to enhanced morbidity and mortality [1–4]. Although the development of atherosclerosis has been shown to be accelerated in this disease [5, 6], recent observations indicate that novel risk factors, such as hypertension, diabetes, hypercholesterolaemia and smoking failed to account for the full cardiovascular (CV) risk [7–9].

These observations have led to efforts directed at identifying novel risk factors for CV disease in SLE: pathogenic anti-endothelial cell (EC) antibodies [10], increased EC apoptosis [11] and a dysbalance of HSP and platelet-activating factor acetylhydrolase [12] are only some of the factors that have been associated with the altered cardiovascular morbidity and mortality in SLE during the last few years.

Endothelial progenitor cells (EPCs), derived from haematopoietic stem cells asahara, represent a population of CD34, VEGF-receptor-2 [VEGFR-2, kinase insert domain receptor (KDR)], AC133-positive cells [13] that contribute to new blood vessel formation (vasculogenesis), since they have the ability to differentiate into endothelial cells. Low levels of circulating EPC correlate with several CV risk factors for coronary artery disease [14] constitute a surrogate marker for vascular dysfunction and the CV risk [15], and have been shown to serve as a predictor for cardiovascular events in a recent outcome study [16]. However, acute ischaemic events, like myocardial infarction [17] or vascular injury, were found to be associated with an increased mobilization of EPC [18] and hypoxia constitutes a potent stimulus for EPC recruitment [19]. Moreover, several drugs, like statins [20], PPAR-γ agonists [21] and erythropoietin (Epo) [22] have been shown to increase EPC differentiation. Circulating EPCs have also been shown to accumulate within the atherosclerotic plaque [23]. It has been suggested that this might constitute a repair process [24]. Therefore, the atherosclerotic plaque is an area of preferential EPC migration in patients suffering from CV diseases. Moreover, it has recently been shown that circulating EPCs are decreased in patients suffering from preclinical atherosclerosis [25].

With respect to all these findings and the current knowledge of EPC biology, it can be assumed that individuals with reduced levels of circulating EPCs have an enhanced CV risk.

On the other hand, a depletion of EPC has been observed in chronic inflammatory diseases such as active RA [26] and SSc [27], as well as both types of diabetes mellitus [28, 29]. These findings are in line with both the increased CV risk in patients with chronic inflammatory conditions [30] and the increased CV risk conveyed by a reduction in circulating EPCs. Interestingly, in patients with RA circulating EPC levels can be normalized by treatment with short-term, intermediate doses of glucocorticoids (GCs) or TNF inhibitors [26].

In the light of the enhanced CV risk in SLE and the insufficient insights into the pathways underlying this propensity, we hypothesized that abnormalities of circulating EPCs may be present in SLE patients.

Patients and methods

Patients

EPCs were quantified in 31 female SLE patients, fulfilling the ACR criteria [31] and in 14 healthy age-matched female controls (HCs) (Table 1). None of the patients had a history of coronary artery disease, myocardial infarction or cardiac insufficiency, which could have influenced the EPC quantities [32]. None of the patients received haemo- or peritoneal dialysis or had undergone kidney transplantation, which could have influenced the EPC counts [33, 34], and the mean creatinine levels were 0.94 ± 0.03 mg/dl (range: 0.58–1.64 mg/dl). None of the patients received recombinant Epo, known to mobilize EPC [35].
At the time of the patients’ visit at our outpatient clinic, blood was drawn to measure blood chemistry, blood count, antibodies to dsDNA and phospholipid as well as C3 and C4, Epo and thrombopoietin (Tpo) serum levels. Clinical assessments included physical examination, measurement of blood pressure and SLEDAI, ECLAM and National Institutes of Health SLE Index Score (SIS) lupus activity score [36]. The results obtained were related to disease activity, organ manifestations and therapies. The local ethics committee approved the study, and patients and HCs provided informed consent for this research.

### Endothelial progenitor cells

Several methods were used for quantification of EPC.

**Flow cytometry analysis.** FACS analyses of EPCs were performed as described previously [14, 26]. Briefly, 100 μl of peripheral blood was incubated with a biotinylated mAb against human KDR (Becton Dickinson, San Jose, CA, USA), followed by staining with streptavidin-RPE-Cy5 conjugate (DakoCytometry, Glostrup, Denmark). Samples were then incubated with FITC-conjugated CD34 (BD Pharmingen, San Diego, CA, USA) and phycoerythrin (PE)-conjugated CD133 (Miltenyi, Bergisch Gladbach, Germany) antibodies. Control stainings were performed with isotype-matched antibodies. Incubation was followed by lysis of red cells and fixation with BD Lysing solution. Acquisition was performed on a Becton Dickinson FACScan flow cytometer and included 300,000 events per sample. Cells positive for CD34/KDR/AC133 within the lymphocyte population were characterized as EPC (Fig. 1).

**Colony forming unit assay.** To detect EPCs by another distinct method, circulating EPCs were also quantified in 13 patients and 12 HCs using the colony forming unit (CFU) assay as described by Hill and colleagues [15]. For this purpose, 5 × 10⁶ isolated peripheral blood mononuclear cells (PBMCs) were plated on fibronectin precoated six-well plates and cultured in medium 199 (Sigma) containing 20% fetal calf serum and penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA) (growth medium) for 48 h. Non-adherent cells were then recollected and 1 million cells/well were replated onto fibronectin-coated 24-well plates (Nunc, Roskilde, Denmark) and cultured in duplicate samples for 7 days in growth medium that was changed every 3 days. CFU, characterized by a central cluster surrounded by emerging cells, were then counted in each 24-well plate. Cells of these colonies express CD31 and KDR [15].

Since patients were treated with various immunomodulating agents, the question arose to which extent potential differences seen between patients with certain therapies were treatment related. We have previously shown that low-dose GC therapy did not have an influence on EPC levels, whereas high-dose GC did [26, 37]. For reasons described in the Results section, we evaluated the effects of chloroquine on CFU generation by assessing colony formation in eight SLE patients and five HCs in the presence or absence of 1, 5, 10, 15 and 25 μM chloroquine. Similar concentrations have been found in vivo in the course of chloroquine therapy [38]. After resuspension, we also evaluated cell death and apoptosis using annexin V (Alexis, San Diego, CA, USA) and 7-aminoactinomycin D (7-AAD) (Calbiochem, San Diego, CA, USA) staining and flow cytometry.

### EPC adhesion assay

To test for adhesion of EPC to human umbilical vein endothelial cells (HUVEC), EPCs were expanded as described by Tepper et al. [39]. Then, 5 × 10⁴ EPCs that had been preincubated with dioctadecyltetramethylindo-carbocyanine-perchlorate (DiI) at a concentration of 2.5 μg/ml (Invitrogen) were seeded into chamber slides with confluent HUVEC (Promocell, Heidelberg, Germany) and incubated at 37°C. After washing the chamber slides three times with PBS, cells were fixed and incubated for 10 min with TO-PRO CA, USA) and 7-aminoactinomycin D (7-AAD) (Calbiochem, San Diego, CA, USA) staining and flow cytometry.

#### Circulating angiogenic cell migration assay

Circulating angiogenic cells (CACS), which are closely related to EPCs [40, 41], can be functionally assessed. An important aspect of the function of such precursor cells is their migratory behaviour. To test this behavior in SLE patients and controls, CAC migration was determined in nine SLE patients and six HCs using Boyden chambers according to an established method [14]. Briefly, PBMCs were isolated by density gradient centrifugation on Ficoll-Paque (Amersham Biosciences, Freiburg, Germany); 4 × 10⁶ PBMCs/well were plated on 24-well culture plates (Nunc) coated with fibronectin in 1 ml EBM-2 medium (Cambrex Bio Science, Walkersville, MD, USA). After 4 days, non-adherent cells were removed by washing twice with PBS, and adherent cells were then incubated with 1 ml EBM-2 medium. After three more days, adherent cells that represent the CAC population [14] were detached using trypsin/EDTA in PBS, harvested by centrifugation and counted. Cells were then resuspended in 100 μl EBM-2 medium and put into a transwell system with fibronectin precoated 24-wells (4 × 10⁴ cells/well). The lower chamber of the 24-well plate was filled with 600 μl EBM-2 medium, enriched with human VEGF (R & D Systems, Minneapolis, MN, USA) at a concentration of 50 ng/ml. After an incubation period of 24 h at 37°C, the transwell filters were fixed with 4% paraformaldehyde for 10 min, washed with PBS and incubated with 4', 6-diamidino-2-phenylindol (DAPI) for 5 min. Filters were then washed and stained cells were counted manually in three random microscopic fields.

### ELISA

The following molecules were determined in the sera of patients and controls by ELISA technique according to standard procedures: TNF (Biosources, Nivelles, Belgium), homocysteine (IBL, Hamburg, Germany), G-CSF, VEGF, basic fibroblast growth factor (FGF), IL-1β, IL-6 (all purchased from R & D Systems).
The lower limits of detection were as follows: TNF 3.0 pg/ml, G-CSF 0.8 pg/ml, VEGF 5.0 pg/ml, basic FGF 0.22 pg/ml, IL-1β 1.0 pg/ml, IL-6 0.7 pg/ml and homocysteine 2.0 μmol/l.

Statistical evaluation

Statistical evaluations were performed using SPSS for Windows v 8.0 (SPSS Inc.). Data were evaluated using normality test, equal variance test and Student's t-test. Data not normally distributed were analysed using non-parametric methods. All data are presented as mean ± S.E.M.

Results

Circulating EPC levels are normal in SLE

EPC levels in SLE patients as analysed both by FACS and by CFU assay were not significantly different in SLE when compared with that in HCs. SLE patients showed a mean of 0.045 ± 0.006% CD34+/KDR/AC133 positive cells within the lymphocyte population and a mean of 18 ± 3 colonies per well as detected by the CFU assay. Similar levels were observed in healthy women both by FACS (0.036 ± 0.007%, P = 0.34, Fig. 2A) and by the CFU assay (15 ± 2 counts/well, P = 0.60, Fig. 2B). The CV risk profile with regard to smoking, diabetes, statin therapy and hypertension did not differ significantly between the patient group and the healthy controls (Table 1). Also, we found no significant differences in circulating EPC levels between SLE patients with and those without CV risk factors (data not shown).

Our finding of normal circulating EPC levels in SLE contrasts other studies that described decreased EPC levels in SLE [42, 43]. However, this might, at least partly, be due to the analysis of different surface markers and the fact that SLE is a heterogeneous disease with different organ manifestations and treatment approaches.

Indeed, when looking at subsets of SLE patients, those with a history of renal disease tended to have higher circulating EPC levels than those without a history of renal lupus (0.058 ± 0.012% vs 0.036 ± 0.005%, 0.05 < P < 0.1, Fig. 2C), possibly related to the increased levels of Epo observed in patients with renal disease (see subsequently); Epo is known to mobilize EPC [35].

No other organ involvement, including RP, which has been described to be associated with altered EPC levels in SSc [27],
Chloroquine were evaluated on CFU isolated from HCs (30 quartently). Similar results were obtained when the effects of reduced at 15/22 chloroquine monotherapy (0.024/22 0.008%), appeared to affect the levels of circulating EPC, i.e. AZA (0.048 ± 0.001%) or mycophenolate mofetil (0.043 ± 0.008%), appeared to affect the levels of circulating EPCs.

**Patients treated with chloroquine exhibit low EPC counts**

SLE patients receiving immunomodulatory therapy of any kind (with the exception of GC monotherapy) had significantly lower EPC levels than those without such treatment (0.035 ± 0.005% vs 0.060 ± 0.011%,  P < 0.05). In-depth investigation within the group of patients receiving immunomodulatory drugs showed evidence for this difference being primarily due to patients treated with chloroquine monotherapy (0.024 ± 0.008%, n = 8,  P < 0.05 vs HC, Fig. 2D), even though disease activity did not significantly differ among these groups. In fact, none of the other treatment regimens, i.e. AZA (0.048 ± 0.001%) or mycophenolate mofetil (0.043 ± 0.008%), appeared to affect the levels of circulating EPCs.

**Chloroquine decreases colony formation in vitro**

To address the possibility that the underlying cause for the observation of EPC reduction in chloroquine-treated patients was a direct effect of this drug on EPC, we performed a series of in vitro experiments exposing EPC colony formation to various concentrations of chloroquine. Interestingly, cells from patients as well as HC exhibited a significant decrease in colony formation in the CFU assay when chloroquine was added to the cultures (Fig. 3). In SLE, we observed a slight reduction in CFU formation already at 5/22 μM and significantly lower CFUs compared with medium alone when cultures were exposed to 10/22 μM (10 ± 4,  P < 0.01 compared with medium alone: 22 ± 2); this was further reduced at 15/22 μM (4 ± 3,  P < 0.001) and 25/22 μM (2 ± 1,  P < 0.001), although these latter doses may have been toxic (see subsequently). Similar results were obtained when the effects of chloroquine were evaluated on CFU isolated from HCs (30 ± 6 with medium only), where the trend towards decreases in CFU started to be seen at 10/22 μM and a significant decrease of CFU formation was observed at doses of 15/22 μM (5 ± 3,  P < 0.05) and 25/22 μM chloroquine (0 ± 0,  P < 0.001).

Testing for early apoptotic and dead cells among the colonies counted revealed that there was no significant effect on healthy control CFUs at 1–15/22 μM, while at 25/22 μM there was significant cell death. Likewise, compared with medium alone there was no significant effect of chloroquine on SLE CFU up to 10/22 μM, while cytotoxic effects were discerned from doses of 15/22 μM and higher; thus, SLE CFU appeared to be somewhat more sensitive to toxic effects of high doses of chloroquine than control cells. Nevertheless, at the lower doses found to significantly inhibit CFU formation, there was no difference in apoptotic or necrotic cells when compared with medium alone. Thus, the ex vivo observation of reduced CFU counts in patients treated with chloroquine appears to be due to a direct inhibitory effect of this antimalarial drug on EPC.

**Impaired adhesion of EPC from SLE patients to HUVEC**

Before circulating EPCs undergo transendothelial migration and contribute to vasculogenesis, they adhere to the vascular wall and migrate into the tissue. We therefore tested the capacity of EPCs from SLE patients and HCs to adhere to confluent HUVEC. As shown in Fig. 4A, the percentage of EPC adhering to HUVEC was significantly lower when EPC from SLE patients were used compared with EPC from HCs (19.2 ± 3.5% vs 36.6 ± 5.2% EPC/high power field,  P < 0.02). Figure 4B and C show a representative image of the adhesion assay. HUVEC are stained with TO-PRO (blue), EPC with Dil (red).

**Migratory activity of CAC isolated from SLE patients**

Migration of endothelial progenitor cells through the vascular wall is important for vasculogenesis i.e. the neovascularization of tissues with an increased demand of oxygenation. Since EPC levels among SLE patients were similar to those of controls despite
the described higher risk for CV events, we wondered if other mechanisms involving EPC might be altered. To this end, we evaluated the migration behaviour of EPC. In fact, counting the migrated CAC in 24-well plates after transmigration revealed that CAC from SLE patients had a decreased migration behaviour compared to controls. We found a mean of 56\%/C6 migrated CAC/over random microscopic field in SLE, while more than twice as many CAC from HC migrated (121\%/C6 migrated CAC/over random microscopic field; \(P < 0.02\), Fig. 4D). This indicated that EPC from SLE patients exhibit a significantly decreased migratory capacity. The decrease in circulating EPC levels among chloroquine treated patients was not compensated by a better migration behaviour, since subanalysis of these patients revealed no significant difference in migratory capacity compared to other patient subgroups and even numerically lower numbers of migrating cells.

Serological testing
As demonstrated in Table 2 and in accordance with the literature [44], SLE patients have higher TNF levels than HC (54 ± 4 pg/ml vs 4 ± 4 pg/ml, \(P < 0.0005\)). Many of the other cytokines and growth factors tested were not detectable in HC, but were detectable in SLE (Table 2). Patients with a history of renal involvement had significantly higher Epo levels than those
without kidney involvement \((20 \pm 4\ \text{vs}\ 11 \pm 1\ \text{mU/ml,}\ P < 0.02)\), possibly due to the impaired Epo response known to be associated with the disease itself \([45]\). However, no correlation between Epo and EPC in both subgroups of patients could be observed.

Interestingly, although their VEGF levels were mostly within normal range, we observed a significant correlation of VEGF serum levels of SLE patients and circulating EPC levels as detected by FACS \((r = 0.383,\ P < 0.05,\ \text{Fig. 5})\).

The mean homocysteine level in the SLE patients was slightly elevated \((13.8 \pm 4.3\ \mu\text{mol/l, upper limit of the normal range: 12.4}\ \mu\text{mol/l})\). Furthermore, we observed correlations of homocysteine with C3c \((r = 0.75,\ P < 0.001)\), haemoglobin \((r = 0.52,\ P < 0.05)\) and Epo \((r = 0.58,\ P < 0.05)\), but not with EPC levels or migration behaviour.

### Discussion

The data presented reveal two novel aspects that differentiate SLE from other chronic inflammatory diseases: first, SLE is associated with normal circulating EPC levels and second and most importantly, the adhesion capacity as well as the migration behaviour of EPC from SLE patients is significantly impaired. In contrast, in many other diseases associated with chronic inflammation, such as RA, SSc, diabetes mellitus and chronic obstructive pulmonary disease (COPD) or in the course of haemodialysis, a decrease of EPC levels has been observed \([26, 27, 39, 46, 47]\) and an inverse correlation between disease activity and the quantity of circulating EPC has been described \([26]\). However, in these diseases there is an adequate acute-phase response, while this has been reported to be blunted in SLE \([48]\).

The two observations related to CRP, namely that SLE patients often exhibit normal CRP levels despite active disease, which was also the case in our study cohort (Table 1), and that CRP reduces EPC survival, differentiation and function \([49]\), are in line with the present data on normal EPC levels in SLE, although it must be assumed that additional factors may play a role.

The fact that our results showing normal circulating EPC levels contrast previous observations \([42, 43]\) requires further discussion. These two publications describe a reduction in EPC levels in patients suffering from SLE. Methodological differences and differences in patient populations may underlie the discrepancy to our findings. First, in both these publications the early EPC specific marker CD133 (or also AC133) was not assessed. However, staining for these two markers is regarded to be pivotal for EPC immunophenotyping \([50]\), since cells positive for VEGFR-2 and CD133 have functional properties of EPC \([13, 51, 52]\). Second, the population studied here comprised mostly whites whereas the study of Lee et al. involved African Americans, whites and others to nearly equal parts \([43]\).

Interestingly, in that study the ethnic distribution among controls did not correspond to that among the patients; there might be differences in EPC levels between various ethnic groups. Third, in that study, almost 80% of the SLE patients received antimalarials, all of them having inactive disease for at least 1 yr, and eight of them, i.e. over 50%, were receiving antimalarials at the time of the study.

Thus, given all the data provided in the present study and in view of the methodological, population and therapeutic issues related to the data published hitherto, we conclude that the levels of EPC, as characterized by VEGFR-2 and CD133 as well as colony forming assays, are in the normal range in SLE patients, with the exception of those receiving antimalarial therapy.

After differentiation in the bone marrow, EPCs are released into the circulation and migrate into tissues with an increased demand for blood supply where they contribute to vasculogenesis \([53]\). Since SLE is known to be associated with an increased CV risk, it was rather surprising that EPC levels in the peripheral blood of these patients were unaffected. This also pertained to patients with phospholipid antibodies who are known to have early atherosclerotic changes \([54]\); they had EPC levels in the range of patients without phospholipid antibodies, and also their EPC migration behaviour did not differ from that of other patient populations (data not shown). However, the relatively small number of patients studied with phospholipid antibodies limits the stringency of this conclusion.

Interestingly, the migration as well as the adhesion capacity of EPC isolated from SLE patients turned out to be significantly impaired compared with those of healthy individuals. Adhesion to the vascular wall and migration through the endothelium contribute importantly to vasculogenesis, one of the major functions of EPC \([53]\). Thus, the impaired functional activity of EPC rather than their number may be an important factor contributing to the increased CV risk associated with SLE, suggesting a relative EPC deficiency in tissues in need for EPC. In fact, the normal EPC levels in SLE patients may even be a consequence of the decreased attachment to the vascular wall and the hampered migration behaviour, since an increase of EPC within the peripheral blood is to be expected when EPC migration toward the perivascular space is impaired. Therefore, it is conceivable that the total ‘active EPC pool’ is reduced in SLE.

In the course of this study, we made another interesting observation. Patients receiving antimalarials exhibited significantly lower EPC levels when compared with controls and other lupus patients, whether treated with immunomodulating drugs or not. Subsequent in vitro experiments revealed that chloroquine, at concentrations attainable in vivo, inhibited colony formation both in SLE patients and HCs. The reduction in circulating EPCs was not compensated by an enhanced migration behaviour, rather the proportion of migrating cells was numerically even lower in this compared with other subgroups of SLE patients. Although it is well known that treatment of mild SLE with immunomodulating drugs reduces the cardiovascular risk associated with the

---

### Table 2. Serum levels of the cytokines and growth factors tested

<table>
<thead>
<tr>
<th>HC</th>
<th>SLE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pg/ml)</td>
<td>99 ± 27</td>
<td>99 ± 10</td>
</tr>
<tr>
<td>bFGF (pg/ml)</td>
<td>n.d.</td>
<td>143 ± 1</td>
</tr>
<tr>
<td>TNF (pg/ml)</td>
<td>4 ± 4</td>
<td>54 ± 4</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>n.d.</td>
<td>54 ± 21</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>n.d.</td>
<td>84 ± 20</td>
</tr>
<tr>
<td>G-CSF (pg/ml)</td>
<td>n.d.</td>
<td>71 ± 12</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>n.d.</td>
<td>13.8 ± 4.3</td>
</tr>
</tbody>
</table>

n.d.: not detectable; n.do.: not done; n.s.: not significant.
disease [55], it has been reported that besides their beneficial effects in SLE antimalarials can exhibit cardiotoxic properties [56]. Whatever the underlying cause of that toxicity, we add here another possible aspect of adverse cardiovascular effects of antimalarials in SLE. Taken together, we hypothesize on the possibility of disparate effects of antimalarials in SLE clinically on the one hand and on EPC behaviour and biology on the other hand. However, given that one of the limitations of our study is the relatively small number of patients investigated and that the present observation is the first of its kind, the findings need further elucidation and validation. Therefore, there is a need for further studies with particular emphasis on antimalarials to better understand the potential impact of the present findings.

Our observation of a tendency towards increased EPC levels in patients with renal lupus also deserves further consideration. Generally, renal insufficiency is known to deplete EPC within the circulation [33], but all patients with a history of nephritis studied here had normal or only slightly elevated creatinine levels at the time of EPC determination. However, they showed significantly elevated Epo levels when compared with patients without renal disease. In fact, although the altered Epo production/secretion in SLE patients often fails to increase erythropoiesis due to impaired marrow Epo response [57], Epo mobilizes EPCs, which could be an explanation for the increased EPC counts in these patients.

Homocysteine is known to be an important CV risk factor in SLE [58] and has also been shown to be increased in our patient cohort, further supporting the notion of an increased CV risk. However, there was no apparent correlation between high homocysteine and low EPC levels, indicating that the risks conveyed by these two factors are independent. The finding of a positive correlation of C3c and homocysteine levels suggests an inverse correlation of homocysteine levels with disease activity and therefore that homocysteine may not be the primary culprit in SLE-associated atherosclerosis. Further studies will have to elucidate the relation between the complement system and homocysteine.

VEGF represents one of the most potent stimuli for EPC mobilization in general [59]. Our observation of a positive correlation between VEGF and circulating EPC levels among SLE patients indicates that recruitment of EPC is not totally compensated by VEGF. These findings might at least partly be responsible for the altered cardiovascular risk known to be associated with the disease.

Acknowledgements

The authors are grateful to Barbara Dekan and Brigitte Meyer for excellent technical assistance.

Disclosure statement: The authors have declared no conflicts of interest.

References