Normal levels and function of endothelial progenitor cells in patients with psoriatic arthritis

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Abstract  Endothelial progenitor cells (EPCs) are a population of bone marrow derived cells which have been attributed with the ability to migrate into areas of tissue ischemia and to possess reparative qualities. EPCs have been shown to be decreased in level and function in various inflammatory disorders. Psoriasis and psoriatic arthritis are associated with an increase in cardiovascular morbidity. The aim of the study was to investigate the number of EPCs among patients suffering from psoriasis and psoriatic arthritis. Patients suffering from active psoriasis and psoriatic arthritis were recruited as well as healthy controls. Disease activity was assessed with the DAS-28, BASDAI and PASI scores. Peripheral blood mononuclear cells were isolated and EPC numbers evaluated by FACS analysis using the CD34/133 and CD34/KDR. No significant difference was found between numbers of EPCs between healthy controls, patients with psoriasis and psoriatic arthritis. A significant correlation was found between levels of VGEF and the BASDAI score. The results of the current study do not support a significant role for EPCs in the pathogenesis of psoriasis and psoriatic arthritis.

Keywords  Psoriatic arthritis · Psoriasis · Endothelial progenitor cells (EPCs) · VGEF

Background  Endothelial progenitor cells (EPCs) are a population of bone marrow derived cells characterized by expression of CD34 and VGEFR-2. These cells, which are estimated to constitute about 0.01% of peripheral mononuclear cells, possess the capacity to proliferate, migrate and differentiate into mature endothelial cells. Characterized by their capacity to transform into mature endothelial cells, EPCs participate in ongoing endothelial repair, while an imbalance between endothelial damage and repair may lead to an increase in cardiovascular events [1]. EPCs are mobilized from bone marrow and migrate to areas of ischemia and or damaged endothelium, participating in angioneogenesis. Thus, EPCs may possess a reparative function, involved in overcoming vascular damage and reducing cardiovascular risk. Levels of EPCs have been shown to increase in conditions such as unstable angina compared to stable angina [2] and decrease in patients undergoing in-stent restenosis compared to those not undergoing such injury, emphasizing the potentially protective effect of these cells [3]. Decreased levels of EPCs have been demonstrated among rheumatoid arthritis patients [4], another systemic inflammatory disorder associated with increased cardiovascular morbidity and mortality; we have previously demonstrated an increase in EPC levels following treatment with the anti-TNFα agent infliximab in rheumatoid arthritis [5].
Psoriasis is a chronic dermatological disorder characterized by epidermal cell proliferation and hyperkeratosis, infiltration of the skin by inflammatory cells, neoangiogenesis, thickening of skin and scaling [6]. Neoangiogenesis is an early stage in the pathogenesis of psoriasis and endothelial cells play an important role in development of the psoriatic plaque. TNFα is a central pro-inflammatory cytokine in the pathogenesis of psoriasis and of psoriatic arthritis. It is secreted in large amounts by activated T cells in psoriatic lesions resulting in chronic inflammation, tissue damage and hyperplasia [7]. TNFα induces secretion of VGEF, a major regulator of angiogenesis [7, 8]. Thus, anti TNFα treatment, e.g. etanercept, possesses the ability to decrease angiogenesis in psoriatic lesions in concordance with the improvement in patients’ clinical condition [8, 9].

Endothelial progenitor cell level and activity is considered as an indicator both of levels of inflammation, levels of vascular damage as well as reflecting reparative potential [10–12]. Various techniques have been described for evaluation and measurement of EPCs. These cells can be isolated in cell culture and their levels inferred through their ability to form characteristic colonies. This technique has been utilized in a series of studies in which EPC levels were evaluated in various pathological conditions [1–3].

On the other hand, multiple studies have made use of FACS sorting to identify and characterize EPCs, based on the expression of specific cell surface makers including CD133, CD34, KDR and VGEF-receptor-2 [4, 13–16]. This method, which has been used in the current study, has significant advantages regarding accuracy.

The level and function of EPCs have not been previously evaluated in psoriatic arthritis. This information has potential significance regarding the pathogenesis of both psoriasis and psoriatic arthritis and could indicate a novel therapeutic avenue for these disorders. Thus, the objective of the current study was to evaluate the levels and function of EPCs among patients suffering from psoriatic arthritis while assessing the correlation between these levels and both clinical parameters of disease activity as well as standard measures of inflammation such as CRP as well as levels of VEGF, a central regulator of angiogenesis.

Methods

The study was conducted after approval of the institutional Helsinki committee and written informed consent was obtained from all participants. Psoriatic arthritis patients were recruited among patients attending a referral rheumatology clinic. Patients with either symmetrical peripheral involvement, asymmetrical peripheral involvement or axial involvement were recruited. Healthy normal controls were recruited among the hospital staff. Exclusion criteria included known cardiovascular disorders, including ischemic heart disease or prior event of CVA, diabetes and active malignancy. Demographic data were recorded as well as information regarding medication use, including anti-inflammatory agents (e.g. NSAIDS), statins etc. Use of anti TNFα medications was documented.

Clinical assessment

Patients underwent clinical evaluation at time of recruitment. Dermatological involvement was documented using the PASI score and articular involvement was documented using a complete 66 joint examination. Axial involvement was evaluated by use of the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) [17], Schober test, lateral spinal flexion, chest expansion and cervical rotation. Dactyliitis was documented.

Based on the clinical characteristics, patients were divided into groups with high and low disease activity. This distinction, which was subsequently utilized for analysis of results, was performed separately based on each clinical parameter. Thus, patients were divided based on the DAS-28 to groups with scores above and below a value of 3.2. Based on the BASDAI patients were divided into groups with scores above and below a value of 3.5. Similarly patients were divided based on the PASI results into groups with values above and below a value of 5.

EPC isolation

Blood samples were acquired upon recruitment from all patients. Mononuclear cells were isolated by Ficoll density gradient centrifugation and separate samples were retained for subsequent evaluation of levels of CRP and VEGF. A total of 1.5 × 10^6 cells were taken for FACS analysis.

FACS analysis was performed with utilization of the following three markers:
1. Phycoerythrin anti-CD34 (IQ products)
2. Allophycocyanin anti-VEGF receptor-2 (KDR, R&D Systems, Minneapolis, MN)
3. FITC-anti CD 133 (R&D systems).

Cells were incubated with the marker for 30 min in the presence of 100 μL fluorescence-activated cell sorter staining buffer (FACS buffer) (phosphate-buffered saline and 2% fetal calf serum) at 4°C. Subsequently cells were washed with phosphate buffered saline (PBS) and fixed with formaldehyde for up to 24 h prior to FACS reading.

FACS analysis was performed with use of Calibur; Becton Dickinson equipment and CellQuest software (BD Bioscience). A total of 80,000 cells were counted at each FACS reading (Fig. 1).
Evaluation of VGEF and CRP levels

Levels of VGEF were determined by use of a commercial ELISA kit (Biosource Camarillo, CA). High sensitivity CRP was determined by ELISA.

Statistics

Comparison between the groups with regard to demographic (gender, age), risk factors (diabetes mellitus, hypertension, hyperlipidemia, smoking, etc.) and clinical factors (EPC numbers, VGEF, CRP etc.), was performed using unpaired Student’s t tests, Mann–Whitney and Chi-square tests, as applicable.

ANOVA was performed for each parameter as described in the results. Spearman’s correlation coefficients were calculated in the groups to study the relationship between CD34/133 and CD34/KDR cells and disease activity. The statistical significance level was set to 0.05 and the SPSS (Chicago, IL) for Windows software, Version 13.0 was used for the analysis.

Results

Table 1 presents demographic data regarding patients in the three groups: group 1—healthy controls \((n = 16)\); group 2—psoriatic patients with no articular involvement \((n = 10)\) and group 3—psoriatic arthritis patients \((n = 22)\).

As is evident from the data presented, the groups did not significantly differ regarding basic demographic characteristics (age, gender, BMI and background medical history).

It must be pointed out that the proportion of smokers was significantly higher among controls and psoriatic patients (groups 1 and 2) compared with group 3 (psoriatic arthritis patients). It is also noteworthy that patients treated with anti-TNFα were found only in group 3.

Evaluation of EPC levels among the 3 groups

Table 2 presents the proportion of cells which stained positive for the abovementioned FACS markers. These cells represent the population of EPCs.

Statistical analysis of these results, using ANOVA calculation, shows the absence of any significant difference between the three groups.

As noted above, in addition to comparing the groups with regard to levels of EPCs, comparison was performed regarding levels of VGEF and CRP. Table 3 presents levels of these two parameters between study groups.

Statistical analysis of these results by use of ANOVA demonstrated no significant difference except for higher levels of CRP among patients with psoriatic arthritis compared to groups 1 and 2 (as expected).

To further characterize the possibility of a difference between groups differing on clinical grounds, further analysis was carried out by subdividing patients into groups with high and low disease activity based on the scores obtained on the DAS-28 scale, using a cutoff of 3.2, based on the BASDAI—using a cutoff of 3.5 and based on the PASI using a cutoff of 5. These groups were once again compared regarding levels of EPCs, VGEF and CRP. The results of

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**Fig. 1**  FACS analysis of CD34 cells

**Table 1**  Clinical parameters of patients in three study groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1: controls ((n = 16))</th>
<th>Group 2: skin psoriasis ((n = 10))</th>
<th>Group 3: psoriatic arthritis ((n = 22))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) ± SD</td>
<td>41.69 ± 9.71</td>
<td>48.6 ± 18.6</td>
<td>47.18 ± 8.15</td>
</tr>
<tr>
<td>Male n (%)</td>
<td>4 (25)</td>
<td>6 (60)</td>
<td>12 (54.5)</td>
</tr>
<tr>
<td>Current smoking n (%)</td>
<td>7 (43)</td>
<td>7 (70)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>Medical history n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td>8 (36.4)</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>Clinical parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic therapy n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-TNF</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>12 (54.5)</td>
</tr>
<tr>
<td>MTX</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>7 (31.8)</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td>2 (9.1)</td>
</tr>
<tr>
<td>Statins</td>
<td>0 (0)</td>
<td>1 (10)</td>
<td>3 (13.6)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>NSAID’s</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>6 (27.3)</td>
</tr>
<tr>
<td>BMI</td>
<td>24.4 ± 3.63</td>
<td>27.05 ± 7.09</td>
<td>27.70 ± 5.34</td>
</tr>
</tbody>
</table>
these analyses demonstrated no significant difference in the levels of EPCs, or VGEF among patients with high disease activity and patients with low disease activity based on the abovementioned parameters, although a non-significant trend appeared to exist towards a decrease in the levels of CD34/133 positive cells among patients with high disease activity (high DAS-28 scores). The only (expectable) difference noted was a significantly higher level of CRP among patients with high disease activity, verifying the validity of the clinical classification.

To further evaluate the possibility of a relationship between EPC levels and the clinical data collected for our patient population, Pearson correlation coefficient was calculated for the relationship between the DAS-28, the BASDAI and the PASI and between the levels of CD34/CD133 positive cells, CD34/KDR positive cells as well as the levels of VGEF and CRP. The results, presented in Table 4, showed a significant correlation between BASDAI values and levels of VGEF. PASI levels were significantly correlated with CRP levels. No significant correlations were identified between any clinical index and the levels of CD34/133 or CD34/KDR cells.

**Discussion**

In the current study, no significant difference was found between EPC levels among healthy controls, patients with psoriasis and patients with psoriatic arthritis. As previously mentioned, a number of studies have previously demonstrated the association between EPC levels and/or function and between various forms of inflammation as well as conditions associated with tissue ischemia and cardiovascular morbidity. In addition psoriasis, in its dermatological manifestations, is associated with endothelial proliferation and has been linked with complex alterations in the levels of various inflammatory and angiogenic mediators including VGEF. This protein, which constitutes a major angiogenic regulator, is over expressed in the skin of psoriatic patients [6, 7, 18]. EPCs, characterized by their...
ability to create new blood vessels, have been previously studied among patients with rheumatoid arthritis [4, 5] and additional inflammatory disorders such as inflammatory bowel disease [16]. A number of studies have demonstrated defects in the levels or function of EPCs among patients suffering from these disorders, patients who are classically considered to be at an increased risk regarding cardiovascular morbidity and mortality [19–21]. In view of these results, the assumption has been made that defective EPC function may play a role in the increased cardiovascular morbidity and mortality in these conditions. In this venue it is noteworthy that in rheumatoid arthritis anti-inflammatory treatment can improve the levels and function of EPCs [5] in association with the clinical improvement; anti-inflammatory treatment has also been shown to decrease the cardiovascular risk associated with inflammatory arthritis [19, 22, 23]. In view of this background, in the current study we attempted to test the hypothesis that the levels of EPCs may be impaired among patients with psoriasis and psoriatic arthritis as well. Such a defect would be a possible explanation for the increase in cardiovascular morbidity which is observed in this patient population [19, 24–26]. While evaluating the negative results of the current study a number of points are worthy of notice. First, as is always the case in the event of negative results, the possibility exists that increasing the size of the sample may have exposed more subtle differences between the groups. The trend towards decreased levels of EPCs among patients with high DAS-28 scores may hint towards this possibility. Another confounding factor is related to the demographic characteristics of the control group which included a higher proportion of smokers thus potentially obscuring differences in EPC function between the groups.

On the theoretical levels, it is possible that the increased levels of VGEF (at least at the level of the skin) observed among psoriasis patients may have a balancing effect regarding EPC levels in contrast to other inflammatory disorders, thus resulting in a EPC level within the normal range (although not necessarily of normal function).

Another point to mention is that the patient population examined was heterogeneous with regard to medications. Some of the patients were treated with anti-TNFα medications, known to possess the ability to increase EPC levels and improve their function [27]. This heterogeneity may have further acted as a confounding factor.

Lately some disagreement has arisen regarding the definition and identifying characteristics of EPCs. These cells were previously identified by their ability to proliferate in cell culture and create clusters with a typical morphology [1], while subsequently FACS has been more commonly used to identify these cells by typical cell surface markers. Unfortunately in many cases a lack of correlation was found between the cells identified by different techniques [14, 28]. Recently the claim has been made that the “real” EPC population is different from the cells previously studied, a population described as endothelial colony forming cells (ECFC) [29]. This theoretical lack of clarity causes difficulty in interpretation of EPC studies as in the current case.

**Conclusion**

In the current study we have attempted to characterize the levels of EPCs among patients suffering from psoriasis and psoriatic arthritis using FACS analysis for the identification of the characteristic cell surface markers CD34/133 and CD34/KDR.

In the current study no significant difference was identified between the level of these cells among patients compared to controls and no significant difference was found between patients with high or low disease activity based on accepted clinical indices.

A positive correlation was found between the BASDAI score and the level of VGEF, a central mediator of angiogenesis. These results do not support a role for EPCs in the pathogenesis of cardiovascular morbidity among psoriatic patients.

**References**


