EXTENDED REPORT

Endothelial dysfunction in patients with rheumatoid arthritis is associated with a reduced number and impaired function of endothelial progenitor cells

K Herbrig*, S Haensel*, U Oelschlaegel, F Pistrosch, S Foerster, J Passauer

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See end of article for authors' affiliations

Correspondence to:
Dr K Herbrig, Department
of Medicine III,
Nephrology, Technical
University of Dresden,
Fetscherstrasse 74, 01309
Dresden, Germany;
kayherbrig@web.de and
haensel.s@debitel.net

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Background: Rheumatoid arthritis (RA) is associated with increased morbidity and mortality attributable to accelerated atherosclerosis and cardiovascular events.

Objective: To determine the role played by endothelial progenitor cells (EPC) in the defence system against arteriosclerosis.

Methods: The number and function of EPC in 13 young patients with RA with low disease activity (DAS28 3.5 (0.3)) and 13 healthy control subjects was studied. Endothelial function was investigated by agonist-induced, endothelium dependent vasodilatation measured by the forearm blood flow technique. Migratory activity and adhesion of EPC to tumour necrosis factor α (TNF α) activated mature endothelial cells and components of the extracellular matrix were tested in vitro. Putative precursor populations (CD34⁺, CD34⁺/CD133⁺, and CD34⁺/KDR⁺ haematopoietic stem cells) were measured by flow cytometric analysis.

Results: Acetylcholine-induced, endothelium dependent vasodilatation was reduced by about 50% in patients with RA, indicating endothelial dysfunction, whereas endothelium-independent vasodilatation in response to glyceryl trinitrate was at control level. Significantly reduced numbers of EPC were found in the patients compared with controls. Migratory activity of EPC was decreased in patients with RA. Adhesion to mature endothelial cells after activation with TNF α was enhanced only in controls. The adhesion to matrix proteins and the number of putative precursor cell lineages was comparable in both groups.

Conclusion: Endothelial dysfunction in patients with RA with low grade inflammation is associated with a reduced number and partial dysfunction of EPC. Further studies are needed to explore whether interventions that potentially ameliorate the number and function of EPC also improve endothelial function in these patients.

Rheumatoid arthritis (RA) is associated with increased morbidity and mortality attributable to accelerated atherosclerosis and cardiovascular events.^{1,2} We and others have recently demonstrated endothelial dysfunction, an early sign of atherosclerosis, even in young patients with RA with low grade inflammatory activity.^{3,4} Among diseases associated with an increased cardiovascular risk RA is unique because conventional risk factors (for example, hypertension, diabetes, hyperlipidaemia) cannot be assumed to have a major causative role in this context. It has been proposed that in RA increased levels of circulating inflammatory mediators may cause activation and damage of endothelial cells, which contributes to endothelial dysfunction.⁵ Recent studies have shed new light on the mechanisms of endothelial repair. It has been shown that bone marrow derived endothelial progenitor cells (EPC) participate in the maintenance of the endothelial cell layer.⁶ Hill *et al* reported that the number of EPC may be a surrogate biological marker for vascular function and cumulative cardiovascular risk.⁷ In their study the number of EPC correlated strongly with the Framingham score and the endothelial function of young healthy male volunteers. It has been shown that patients at risk for coronary artery disease have decreased numbers of circulating EPC with impaired activity.⁸ The authors suggested that EPC have a significant role of in patients with coronary artery disease.

Conceivably, an altered number and/or function of EPC contributes to the accelerated arteriosclerosis seen in patients with RA. Hypothetically, chronic inflammation or deficiency of growth factors required for maturation and differentiation may cause putative "EPC dysfunction" in RA. Therefore it might be potentially relevant for the pathogenesis and later treatment development to study whether EPC release from bone marrow and/or functions of ex vivo expanded EPC are altered in patients with RA.

PATIENTS AND METHODS

Characteristics of patients and controls

The study was approved by the institutional review board of our medical centre. All participants gave written informed consent. Thirteen patients with documented RA for a mean (SEM) of 7.7 (2.7) years were selected from the outpatient programme of the division of rheumatology. All patients underwent routine clinical examination. To avoid confounding factors potentially relevant for EPC kinetics or

Abbreviations: Ach, acetylcholine; ANOVA, analysis of variance; DAS28, 28 joint count Disease Activity Score; DiLDL, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine labelled acetylated low density lipoprotein; EBM, endothelial basal medium; EFl, endothelial function index; EPC, endothelial progenitor cells; FBF, forearm blood flow; GTN, glyceryl trinitrate; HCAEC, human coronary artery endothelial cells; HPF, high power field(s); hsCRP, high sensitivity C reactive protein; IL, interleukin; MNCs, mononuclear cells; MTX, methotrexate; PBS, phosphate buffered saline; PI, propidium iodide; RA, rheumatoid arthritis; TNF α , tumour necrosis factor α ; VEGF, vascular endothelial growth factor

*K Herbrig and S Haensel contributed equally.

measurement of endothelial dysfunction, we excluded patients with diabetes mellitus, a past medical history of coronary artery disease, and smokers. None of the patients had been treated with statins previously. Matched healthy volunteers served as a control group. Table 1 shows the characteristics of the study group. Disease modifying anti-rheumatic drugs included methotrexate (MTX) in all and anti-TNF α antibodies in six patients. None of the patients received glucocorticoids. The disease activity of RA was well controlled as demonstrated by the low disease activity score (28 joint count Disease Activity Score (DAS28)).

Forearm blood flow analysis

Studies were performed in a quiet, temperature controlled room (23–25°C) with the subjects resting supine. At the beginning of each experimental session a clinical evaluation was performed and venous blood was collected for measurement of laboratory variables, isolation of EPC, and flow cytometric analysis. The DAS28 tender joint count was assessed in all patients.⁹

Studies were performed as described previously.³ Briefly, infusions of test agents were given into the brachial artery of the non-dominant arm via a 27 G needle. Forearm blood flow (FBF) was measured in both arms by venous occlusion plethysmography. During all recording periods the hands were excluded from the circulation by a wrist cuff inflated to a suprasystolic pressure of 220 mm Hg.

Measurements of baseline FBF were started 20 minutes after arterial puncture. Each dose of agent was given over 5 minutes at a constant rate of 1 ml/min. Between the infusions of different agents we kept a 30 minute control period during which isotonic saline was infused. The study protocol consisted of infusions of graded doses of acetylcholine (ACh; Miochol-E; Ciba Vision, Germering, Germany) 55, 110, and 220 nmol/min and glyceryl trinitrate (GTN; Perlinganit; Schwarz Pharma, Monheim, Germany) 2.2, 4.4, and 8.8 nmol/min.

EPC culture assay

Mononuclear cells (MNCs) were isolated by density gradient centrifugation with Histopaque-1077 (Sigma) from 27 ml of

peripheral blood. Immediately after isolation 1×10^6 MNCs were plated on an eight chamber culture glass slide coated with fibronectin (Sigma) and were maintained in an endothelial basal medium (EBM; CellSystems) supplemented with EGM SingleQuots and 5% fetal calf serum. After 3 days in culture, non-adherent cells were removed; adherent cells underwent cytochemical analysis on day 4.

Characterisation of EPC

To detect the uptake of 1,1'-dioctadecyl-3,3',3'-tetramethyl-indocarbocyanine labelled acetylated low density lipoprotein (DiLDL; Molecular Probes), cultivated cells were incubated with DiLDL (10 μ g/ml) at 37°C for 1 hour. Thereafter cells were fixed with 2% paraformaldehyde for 10 minutes and incubated with FITC labelled UEA-I (lectin, 10 μ g/ml; Sigma) for 1 hour. Cells staining positive for both lectin and DiLDL were judged to be EPC. Their numbers were counted for each well by two blinded investigators. They counted three to five randomly selected high power fields (HPF) for each well. To confirm the phenotype, the expression of surface marker proteins was measured by flow cytometric analysis as recently reported by us.¹⁰

Flow cytometric measurement and analysis

Flow cytometric measurement was performed on a FACS Calibur flow cytometer (Becton Dickinson).

Firstly, a three colour analysis was performed using CD45 FITC (DAKO Cytomation), CD34 PE (BD Sciences), and CD133 APC (Miltenyi Biotec). After the incubation of peripheral blood with the above mentioned antibodies (15 minutes at room temperature), red cells were lysed for 10 minutes with FACS lysing solution (BD Sciences). Thereafter, cells were washed and resuspended in 500 μ l phosphate buffered saline (PBS; Seromed). Measurement was performed after setting a live gate on cells with low side scatter in the dot plot SSC ν CD34. In this gate 50 000 events were registered.

Secondly, on cultured EPC, two colour analyses using VE Cadherin FITC (BenderMedSystems), CD31 PE (Cymbus Biotechnology), and CD146 FITC (Biocytex) were performed. At least 10 000 cells were measured after gating cells in an FSC/SSC dot plot. Fluorescence intensity was analysed using GeoMean.

Thirdly, EPC were characterised with KDR (ReliaTech) after indirect staining using a phycoerythrin conjugated goat antimouse secondary antibody (DAKO Cytomation). Non-matched, isotype-specific antibodies served as controls in each measurement.

Migration assay

Isolated EPC were detached mechanically, harvested by centrifugation, resuspended in 300 μ l EBM (without vascular endothelial growth factor (VEGF)), and counted. 2×10^4 EPC were placed in the upper chamber of a modified Boyden chamber. The chamber was placed in a 24 well culture dish containing EBM and human recombinant VEGF (50 ng/ml; Sigma). After 24 hours of incubation at 37°C, the lower side of the chamber, containing the migrated cells, was washed with PBS and fixed with 2% paraformaldehyde. For quantification cell nuclei were stained with diamidinophenyl indole and counted in three random microscopic fields by two blinded investigators. Measurement was performed in duplicate.

To examine the effect of a microinflammatory environment on EPC bioactivity we cultured EPC from six control subjects and EGM-2 containing 20% human serum pooled from six healthy volunteers or from six patients with RA (instead of 5% fetal calf serum, standard cell culture condition). Thereafter the migration assay was performed as described above.

Table 1 Characteristics of patients with RA and healthy control subjects

Characteristics	Control subjects (n = 13)	Patients with RA (n = 13)
Male	1	1
Age (years)	34.5 (1.4)	33.9 (2.7)
BMI (kg/m ²)	23.1 (0.9)	24.9 (1.6)
Duration of RA (years)		7.7 (2.7)
Blood pressure		
Systolic	123.5 (3.3)	123.0 (4.1)
Diastolic	73.4 (1.8)	75.5 (3.1)
Heart rate (beats/min)	77.6 (2.4)	70.0 (2.1)
DAS28		3.5 (0.3)
hsCRP (mg/l)	1.2 (0.4)	4.0 (1.3) [†]
Interleukin 6 (pg/ml)	1.4 (0.3)	3.3 (0.5) [*]
Haemoglobin (mmol/l)	8.5 (0.2)	8.6 (0.2)
Packed cell volume	0.41 (0.01)	0.41 (0.03)
Leucocytes (GPI/l)	6.6 (0.5)	7.5 (0.5)
Thrombocytes (GPI/l)	252 (24)	318 (22)
HbA _{1c} (%)	5.0 (0.1)	5.1 (0.1)
Homocysteine (μ mol/l)	12.3 (0.7)	10.8 (0.9)
Soluble transferrin receptor (mg/l)	1.1 (0.1)	1.5 (0.1)
Total cholesterol (mmol/l)	4.8 (0.2)	4.5 (0.3)
Triglycerides (mmol/l)	0.94 (0.1)	0.93 (0.2)

Data are means (SEM).

^{*}p<0.003; [†]p<0.05.

BMI, body mass index; DAS28, 28 joint count Disease Activity Score;

hsCRP, high sensitivity C reactive protein; HbA_{1c}, major fraction of glycosylated haemoglobin.

Figure 1 Forearm blood flow (FBF) in response to ACh and GTN in control subjects and patients with RA; * $p < 0.003$ by ANOVA for repeated measurements.

EPC adhesion to matrix molecules

Collagen type IV (100 $\mu\text{g/ml}$), fibronectin (100 $\mu\text{g/ml}$), or laminin (2 $\mu\text{g/ml}$) was coated onto four chamber culture glass slides for 2 hours at 37°C. Wells were blocked with 1% bovine serum albumin in PBS for 1 hour and 1×10^5 EPC were added to each well. Non-adherent cells were removed after 1 hour. Adherent cells were counted in three random microscopic fields by two blinded investigators.

EPC adhesion to endothelial cells

A monolayer of human coronary artery endothelial cells (HCAEC) was prepared 72 hours (in four-chamber glass slides) before 1×10^5 EPC (DiLDL labelled) were added to each well and incubated for 2 hours at 37°C. HCAEC were pretreated for 12 hours with $\text{TNF}\alpha$ (1 ng/ml) or medium.

Non-attached cells were gently removed. Cells were fixed with 2% paraformaldehyde for 10 minutes. Nuclei of all cells were stained by diamidinophenyl indole. EPC and HCAEC were counted in three HPF by two blinded investigators. EPC as a percentage of the total cells for each HPF was used for statistical analysis.

Apoptosis assay

Quantitative determination of cells undergoing apoptosis was carried out using an annexin V apoptosis detection kit (Alexis), according to the manufacturer's instructions.

The percentage of apoptotic cells was measured in EPC (from five healthy volunteers) cultured with and without

MTX (0.1 $\mu\text{mol/l}$, 1 $\mu\text{mol/l}$, 10 $\mu\text{mol/l}$, and 100 $\mu\text{mol/l}$) for 4 days. Cells were stained with annexin V-FITC and propidium iodide (PI) and subjected to flow cytometric analysis. To exclude necrotic cells only annexin positive cells were counted.

Data are given as means (SEM) as a percentage of annexin V^+/PI^- cells (representative of apoptotic cells).

Blood levels of VEGF and interleukin (IL) 6

Blood levels of VEGF and IL6 were measured by a highly sensitive enzyme linked immunosorbent assay (ELISA; R&D Systems), according to the manufacturer's instructions. Samples were checked by serial dilution and measurements were performed in duplicate.

Calculation and statistics

Forearm blood flow studies

In general, each determination of FBF was calculated as the mean of the last five individual FBF measurements. Results are presented as absolute FBF in the infused and non-infused arms. For statistical analysis of the vascular responses to ACh and GTN, we compared the results obtained in the infused arm by two way analysis of variance (ANOVA) for repeated measurements. The comparison of baseline FBF between groups was made by Student's t test. For correlation analyses we calculated an endothelial function index (EFI) as follows: $\text{EFI} (\%) = (\text{FBF (ACh 220 nmol/min)} - \text{FBF (baseline before$

Figure 2 (A) Over 95% of adherent cells are positive for DiLDL and UEA-I (upper right field). (B) The phenotype of cultured cells was studied by flow cytometric analysis of specific marker proteins: CD31, VE-cadherin, and KDR (for endothelial differentiation), and CD146 (mature endothelial cells) was used for exclusion of contaminating adult endothelial cells. Representative images are shown. Plots show specific antibody staining (black) and isotype controls.

ACh)/(FBF (GTN 8.8 nmol/min)–FBF (baseline before GTN) $\times 100$)–100.

The EFI gives the endothelium dependent vasodilatation as a percentage of the endothelium independent vasodilatation. Equal values of both types of vasodilatation result in an EFI of zero.

EPC studies

Comparison of continuous variables was performed using two way ANOVA. Statistical significance was considered to be present at the 5% level. All statistical analyses were performed using the computer software SPSS for Windows 12.0. All data are given as means (SEM).

RESULTS

Forearm blood flow analysis

Endothelial function of the study participants was tested by agonist-induced, endothelium dependent vasodilatation as measured by the FBF technique. ACh-induced vasodilatation was significantly reduced in patients with RA (fig 1), whereas endothelium-independent vasodilatation studied by infusion of GTN was at the control level (fig 1).

EPC characterisation

EPC were characterised as cells dual stained positive for DiLDL and UEA-I; more than 95% of the adherent cells were positive for both (fig 2A). In addition, their endothelial phenotype was confirmed by demonstrating the expression of the endothelial marker proteins vascular endothelium-cadherin (VE-cadherin), CD31, and KDR by flow cytometric analysis after 4 days of culture (figs 2B–D). We found that

45.9 (5.4)% were positive for VE-cadherin, 90.9 (5.9)% for KDR, and 96.9 (1.5)% for CD31. Human coronary artery cells served as positive control cells (VE-cadherin: 69.8 (6.9)%; KDR: 59.7 (7.7)%; CD31: 99.6 (0.2)%).

It is important to exclude relevant numbers of circulating mature endothelial cells contributing to the observed out-grow of EPC from the starting MNC population. CD146 is a marker for mature endothelial cells; our cultured cells stained positive for the endothelial markers mentioned above and negative for CD146. Therefore it is likely that the studied cells are progenitor cells but not circulating mature endothelial cells (fig 2E).

EPC number

MNCs from healthy volunteers and patients were cultured for 4 days, and EPC were characterised and counted as described above. The number of EPC in the culture assay was significantly reduced in patients with RA (230 (29) ν 490 (48) EPC/HPF; $p < 0.001$); fig 3 shows representative fluorescent microscopy results.

We found a positive correlation between the number of EPC and the vascular response expressed by the EFI over the entire study group (fig 3D).

EPC function in vitro

We observed a partial dysfunction of EPC in patients with RA. In detail: migratory activity in response to VEGF was reduced in the patients with RA (50.2 (7.4) ν 88.5 (8.5) EPC/HPF; $p < 0.005$, fig 4A). If MNCs of control subjects ($n = 6$) were cultured in EGM-2 supplemented with pooled serum of patients with RA ($n = 6$) the EPC showed a decreased

Figure 3 Dual stained cells positive for both lectin (green) and DiLDL (red) were judged as EPC and counted for each well. Representative high power fields of a control subject (A) and a patient with RA (B) are shown. (C) Number of EPC/HPF in patients with RA and controls, $p < 0.001$. (D) Positive correlation between EPC count in vitro and endothelial function estimated by endothelial function index in the entire study group.

migratory activity compared with MNCs cultured in EGM-2 supplemented with serum of healthy volunteers (39.8 (5.1) ν 61.4 (5.9) cells/HPF; $p = 0.02$) (fig 4B).

The adhesion of EPC to non-activated HCAEC cultured from MNCs of patients was comparable to the number of adherent cells of the control group (9.7 (1.3) ν 9.2 (1.8)% of total cell number/HPF, $n = 13$). We observed a significant increase in adherent EPC of 88% in control subjects after activation of HCAEC with $\text{TNF}\alpha$ ($p < 0.005$). EPC from patients with RA showed only a mild increase of 20% after $\text{TNF}\alpha$ activation, ($p = 0.5$). The effect of $\text{TNF}\alpha$ activation was proved in six participants in each group. There were no differences between patients and control subjects in the adhesion of EPC to matrix proteins. The highest adherence in both groups was seen when EPC were added to fibronectin coated chamber slides (control: 19.7 (1.6)/HPF; patients with RA: 19.8 (2.9)/HPF), followed by adhesion to laminin (control: 15.8 (1.5)/HPF; patients with RA: 15.5 (3.3)/HPF). Adhesion to collagen type IV coated chamber slides was

found at a level of 13.2 (1.0)/HPF in control subjects and 11.6 (1.2)/HPF in patients.

Number of CD34^+ , $\text{CD34}^+/\text{KDR}^+$, $\text{CD34}^+/\text{CD133}^+$ cells
Some of the putative precursor populations of EPC (CD34^+ , $\text{CD34}^+/\text{KDR}^+$, $\text{CD34}^+/\text{CD133}^+$ cells) were investigated by flow cytometric analysis. We found reduced numbers of each in the patients with RA, but the differences did not reach

Figure 4 (A) Migration of EPC from patients with RA and controls. (B) Migratory activity of EPC from control subjects after culture in EGM-2 supplemented with pooled serum of healthy volunteers or serum of patients with RA. * $p < 0.005$; ** $p = 0.02$.

Figure 5 The EPC count measured in vitro by the culture assay showed an inverse correlation with blood levels of IL6 in the entire study group.

statistical significance. The following data were obtained: CD34⁺ cells: 0.020 (0.003)% in patients with RA *v* 0.027 (0.004)% in controls (*p* = 0.25); CD34⁺/CD133⁺ cells: 0.015 (0.002)% *v* 0.022 (0.004)% (*p* = 0.13); CD34⁺/KDR⁺ cells: 0.005 (0.001)% *v* 0.007 (0.002)% (*p* = 0.5).

Role of MTX-induced EPC apoptosis

To evaluate the potential influence of MTX on apoptosis we analysed the binding of annexin V in EPC from healthy volunteers cultured for 4 days with and without MTX. A significantly increased apoptosis rate was found after incubation of EPC with MTX at 10 and 100 µmol/l compared with cells cultured in MTX free environment (control: 15.7 (1.9); 0.1 µmol/l: 15.7 (3.0); 1 µmol/l: 16.5 (2.2); 10 µmol/l: 21.5 (3.4); 100 µmol/l: 22.5 (3.4) as a percentage of total cells, *p* < 0.01).

Cytokines

Mean blood levels of VEGF were similar in both groups (VEGF: 406.8 (34.6) pg/ml in control subjects *v* 324.3 (27.5) pg/ml in patients with RA). IL6 was raised almost 2.5-fold in patients with RA (1.4 (0.3) *v* 3.3 (0.5) pg/ml, *p* < 0.003). The IL6 blood levels correlated negatively with the number of EPC/HPF in vitro when correlation was calculated over the entire study group (fig 5). No such correlation was found between EPC number and high sensitivity C reactive protein (hsCRP).

DISCUSSION

This study was designed to test the number and function of EPC in young patients with low grade activity of RA receiving standard MTX treatment and with proven endothelial dysfunction. The latter was demonstrated by an impairment of agonist-induced endothelium dependent vasodilatation in this particular group of patients compared with matched control subjects. In the culture assay we found that the total number of EPC was significantly reduced in patients with RA. This result adds to previous findings showing a reduced number of EPC in patients with diseases associated with an increased risk for cardiovascular events—for example, diabetes mellitus types 1 and 2, coronary artery disease, hyperlipidaemia, and chronic renal insufficiency. Moreover, we found a significant correlation between the number of EPC and endothelial function over the entire study group. The count of putative precursor populations (CD34⁺, CD34⁺/CD133⁺, CD34⁺/KDR⁺ cells) detected by flow cytometric analysis was reduced in the patients with RA, but the differences did not reach statistical significance.

The ability to express endothelial marker proteins after ex vivo expansion is also reported for CD34 negative cells. CD14⁺, CD14⁺/VEGFR2⁺, CD14⁺/Tie-2⁺ cells, and mesenchymal stem cells can develop an endothelial phenotype in vitro.^{11–15} Trans-differentiation of monocytes positive for VEGFR2 has been reported, too.^{16, 17} Confirming recent data reported by Kuwana *et al*,¹⁸ our finding may indicate that stem cell lineages other than CD34⁺ stem cells contribute to the generation of EPC. Differences in the cell populations mentioned above, or an altered transdifferentiation of monocytes, may therefore have contributed to the observed reduced number of EPC in patients with RA in vitro.

In addition to the EPC count we also investigated the state of important functional features of EPC in vitro. We found a significantly reduced migratory activity of EPC in patients with RA compared with controls, whereas the ability of EPC to adhere to mature endothelial cells and to components of the extracellular matrix was mainly at the control level. Activation of mature endothelial cells with TNFα increased the adhesion of EPC from healthy subjects but not of EPC from patients with RA. These results are compatible with

partial EPC dysfunction in patients with RA receiving standard MTX treatment.

In principle, there are two possible explanations for the alterations in the EPC number and function seen in patients with RA: firstly, the intake of disease modifying antirheumatic drugs and secondly, the presence of a steady low grade inflammation.

We were therefore interested to determine whether our findings might be explained by MTX-induced apoptosis of EPC. Our in vitro measurements of apoptotic EPC cultured in MTX containing medium demonstrated a small, albeit significantly increased, apoptosis rate compared with control medium. Although this finding is unlikely to explain entirely the reduced number of EPC in patients with RA, this mechanism might be a contributing factor. In this context it would be interesting to study the influence of MTX on haematopoietic bone marrow stem cells, too.

hsCRP and IL6 were increased in the serum of patients with RA, indicating the presence of low grade inflammatory activity. A relationship between the number of circulating angiogenic cells and the inflammatory activity in patients with RA has been recently reported by Grisar *et al*.¹⁹ They found an inverse correlation between the disease activity assessed by the DAS28 and CD34⁺/CD133⁺/KDR⁺ cells in the peripheral blood. We are unable to confirm this particular result. However, our finding of a negative correlation between the IL6 level and the number of EPC/HPF over the entire study group may support the hypothesis that the individual microinflammatory state is related to the number of circulating EPC. Furthermore, it is known from in vitro experiments that CRP attenuates EPC survival and recently, it has been demonstrated that CRP has the potential to decrease the angiogenic function of EPC.^{20, 21}

A further mechanism which may play a part in the reduced number of EPC in the circulation is the homing of circulating EPC to sites of active neovascularisation and inflammation. Rüger *et al* reported recently that EPC are present in the synovial membranes of patients with RA, where they form cell cultures to generate new vessels.²²

In summary, our data show a reduced number and an impaired function of EPC in young patients with RA with low disease activity and proven endothelial dysfunction. Further studies need to explore whether interventions that potentially ameliorate the number and function of EPC also improve endothelial function in these patients.

Authors' affiliations

K Herbrig, F Pistorosch, S Foerster, J Passauer, Nephrology, Department of Internal Medicine III, Technical University of Dresden, Dresden, Germany

S Haensel, Rheumatology, Department of Internal Medicine III, Technical University of Dresden, Dresden, Germany

U Oelschlaegel, Haematology, Department of Internal Medicine I, Technical University of Dresden, Dresden, Germany

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