Review

Circulating Endothelial Cells and Circulating Endothelial Progenitors in Kidney Disease – Victims, Witnesses, or Accomplices?

(circulating endothelial cell / circulating endothelial progenitor cell / endothelium / endothelial dysfunction / atherosclerosis / haemodialysis / end-stage renal disease / kidney transplantation / ANCA-associated vasculitis / angiogenesis / vasculogenesis)

J. ZÁVADA[1], L. KIDERYOVÁ[2], R. PYTLÍK[2], V. TESAŘ[1]

Charles University in Prague, First Faculty of Medicine and General Teaching Hospital: [1]Department of Nephrology, [2]First Medical Department – Clinical Department of Haematooncology, Prague, Czech Republic

Abstract. Nephrologists deal with a host of pathologic conditions involving renal and systemic endothelium. Both in native and transplanted kidneys, often the insult to the renal endothelium initiates the pathogenic process ultimately leading to the loss of organ function. Also, systemic atherosclerosis is accelerated in patients with renal dysfunction. In this review we would like to cover the possible role of CECs and their counterparts - circulating EPCs in the pathogenesis of endothelial dysfunction associated with chronic renal failure, ANCA-associated vasculitis, and progression of chronic renal disease.

Introduction

Nephrologists deal with a host of pathologic conditions involving renal and systemic endothelium. Both in native and transplanted kidneys, often the insult to the renal endothelium initiates the pathogenic process ultimately leading to the loss of organ function. Systemic atherosclerosis is well known to be accelerated in patients with renal dysfunction, and both renal and systemic endothelium is subject to rapid damage in certain disease states, i.e. hypertensive crisis, preeclampsia, or small vessel vasculitis. Endothelial damage ultimately represents a balance between the magnitude of injury and the capacity for repair, and the measurement of the number and function of endothelial cells and their precursors circulating in the peripheral blood is becoming an essential part of the current paradigms concerning endothelial function and turnover. In this review we cover the possible role of these circulating endotheloid cells, i.e. circulating endothelial cells (CECs) and their counterparts – circulating endothelial progenitor cells (EPCs) in the pathogenesis of 1) endothelial dysfunction associated with chronic renal failure, 2) anti-neutrophil cytoplasmic antibodies (ANCA)-associated small vessel vasculitis, and 3) the mechanisms of progression of chronic renal disease.

Endothelium and the kidney

The endothelium is the largest organ in the body consisting of endothelial cells lining every blood vessel. The endothelial monolayer plays a critical role in vascular homeostasis. In addition to forming a physical barrier between the vessel wall and the lumen, endothelial cells secrete a range of compounds which modulate vascular tone, coagulation, cell proliferation, and inflammation.

Endothelial function is an important barometer of overall vascular risk and correlates with established and emerging cardiovascular risk factors. Increasing evidence suggests that uraemia causes endothelial dysfunction (Cross, 2002). Impaired renal function by itself has been proved to be a strong independent risk factor for cardiovascular disease, which largely contributes to the exceedingly high annual mortality rates (15–20 %) seen in conventional haemodialysis (HD) patients (Foley et

Received October 11, 2007. Accepted June 16, 2008.

Supported by the research project of the Ministry of Education, Youth and Sports of the Czech Republic MSM0021620807, grant of the Ministry of Health of the Czech Republic IGA NR 8047/3, and grant from Charles University GA UK 33/2006.

Corresponding author: Jakub Závada, Charles University in Prague, First Faculty of Medicine and General Teaching Hospital, Department of Nephrology, U nemocnice 2, 128 00 Prague 2, Czech Republic. Fax: (+420) 224 962 585; e-mail: jzavada@centrum.cz

Abbreviations: AAV – ANCA-associated vasculitis, ANCA – anti-neutrophil cytoplasmic antibodies, CEC – circulating endothelial cell, EPC – endothelial progenitor cell, GFR – glomerular filtration rate, HD – haemodialysis, RT – renal transplant patient (recipient), SLE – systemic lupus erythematosus, VEGF – vascular endothelial growth factor.

al., 2005). Traditional risk factors such as hypertension and diabetes only partly explain this increase in vascular risk, with the increasing recognition that kidney failure *per se*, through a number of mechanisms, may be directly responsible for the adverse cardiovascular outcome in this vulnerable patient population. Although cardiovascular mortality in renal transplant recipients is significantly lower compared with those remaining on dialysis, still the risk of cardiovascular disease in renal transplant patients (RT) is higher than in general population (Ojo, 2006).

Recent evidence suggests that injury to the renal vasculature may play an important role in the pathogenesis of both early and chronic ischemic acute kidney injury (AKI). It seems that endothelial cell injury participates in the extension and maintenance of AKI by pathways that are related to vascular tone. Furthermore, reductions in microvasculature density may play a critical part in the progression of chronic kidney disease following initial recovery from ischemia/reperfusion-induced AKI (Basile, 2007). There is an emerging view that chronic hypoxia, induced by loss of peritubular capillaries or increased vascular resistance, may result in the development of tubulointerstitial fibrosis in renal diseases of diverse aetiologies, representing a potential common pathway for disease progression (Kang et al, 2002). Even in the normal aging process, it has been shown that the loss of glomerular endothelium is associated with progressive renal impairment. Endothelial cell loss and dysregulated repair have also been found to be of pathophysiologic significance in anti-glomerular basement membrane models of glomerulonephritis (Ohashi et al., 2000). Also, hypertension, diabetes and hypercholesterolaemia, prevailing conditions in nephrology, profoundly affect the endothelium. In systemic autoimmune diseases such as ANCA-associated vasculitis and systemic lupus erythematosus (SLE), endothelial cells in systemic circulation as well as in renal microvasculature are targets of antibody- or immune complex-mediated injury. Finally, in thrombotic microangiopathies such as malignant hypertension, preeclampsia, radiation nephritis, and haemolytic uraemic syndrome, endothelial cell activation and apoptosis may play a major pathogenic role.

Until recently, postnatal vascular repair and regeneration was thought to result exclusively from **angiogenesis**, outgrowth of fully differentiated mature endothelial cells from pre-existing blood vessels. It was discovered that mononuclear cells in peripheral blood have the potential to differentiate into endothelial cells and may give rise to *de novo* **vasculogenesis**, a process hitherto thought only to occur in the developing embryo (Asahara et al., 1997). Both these reparative processes could be defective in patients with renal disease, and there is growing interest in the concept that manipulation of these responses can attenuate the disease process.

Assessment of endothelial function

Because endothelium is relatively inaccessible to direct examination, investigators have concentrated on various surrogate markers of endothelial function.

One approach to the assessment of endothelial functioning relies on the changes of specific plasma markers (such as von Willebrand factor, soluble thrombodulin, tissue plasminogen activator, soluble endothelial protein C receptor, and soluble E selectin); another is by using physiological techniques (such as flow-mediated dilatation after reactive hyperaemia). More recently, an additional method for assessing vascular integrity has been developed: measurement of endotheloid cells (and endothelial microparticles) in peripheral blood. Principal among these endotheloid cell populations are **circulating endothelial cells** (CECs) and **endothelial progenitor cells** (EPCs). The former are thought to originate from sloughing off the vessel wall following some form of pathological insult. The latter are believed to arise from the bone marrow, and to be important in repair following vascular damage.

Methodologies to determine circulating endotheloid cells in blood

In practice, one of the major problems with the quantification of both CECs and EPCs is represented by their low numbers present in the blood, varying between 001% and 0.0001% of mononuclear cells. Improved detection of these rare events can be achieved by combining cell enrichment techniques such as density centrifugation with labelling of these cells with a specific marker. For detection of CECs, most groups use either immunomagnetic beads or flow cytometry, and CD146 is the most popular choice of a marker for CECs. EPCs are commonly detected by flow cytometry counting cells co-expressing endothelial markers such as vascular endothelial growth factor (VEGF) receptor-2 (VEGFR-2 or kinase domain receptor, KDR), CD31, and von Willebrand factor in addition to stem cell or progenitor markers such as CD133 or CD34. Alternatively, EPCs could be evaluated by their capacity to form colonies (colony-forming units) *in vitro*, and to incorporate acetylated low-density lipoprotein and bind lectins (Ingram et al., 2005).

CECs – biomarkers of vascular disease

There is an expanding list of conditions associated with severe endothelial injury and elevated numbers of CECs in peripheral blood. It seems that increased CEC numbers in the blood are the product of a disease process that damages the endothelium. The potential mechanisms of endothelial cell detachment from the vessels are multiple. Different experimental models have documented that denudation of the vessels can be triggered by mechanical injury, defective adhesive properties of the endothelial cells, protease- or cytokine-mediated de-

tachment, or activation of the apoptotic programme (Segal et al., 2002).

Numerous studies in the literature have documented that increased numbers of CECs (by almost any definition) correlate with disease severity, as assessed by standard clinical methods. In cardiovascular disease, the highest numbers of CECs have been found in the blood of subjects with the most severe and acute coronary artery disease (Mutin et al., 1999). Also, the CECs count correlated with the risk of cardiovascular events (Boos et al., 2007), and combined with troponin level, has been proved as an early, specific, independent diagnostic marker for non-ST-elevation acute coronary syndromes (Quilici et al., 2004)). Similarly, the highest CECs have been found in patients with the most severe peripheral artery disease (Makin et al., 2004). In the field of inflammatory and connective tissue diseases, high CEC levels were reported in patients with malignant forms of Mediterranean spotted fever (George et al., 1993); the number of CECs strongly correlated with the severity of small-vessel vasculitis (Woywodt et al., 2003a) and systemic sclerosis (Del Papa et al., 2004). In SLE, a prototypic autoimmune condition in which circulating immune complexes are likely involved in endothelial stimulation and shedding, patients with active disease expressed significantly higher levels of CECs in peripheral blood compared to patients with inactive disease or healthy controls (Clancy et al., 2001).

What could CECs tell us in renal disease?

CECs in end-stage renal disease

Koç et al. (2003, 2005) conducted two studies investigating the role of CECs in HD patients. In the first one he showed that the number of CECs in patients undergoing long-term HD treatment is increased compared to healthy controls. However, even higher numbers of CECs were observed in control groups of patients with hypertension or diabetes and concomitant mild renal dysfunction. Because at least 60% of patients on HD therapy have diabetes or hypertension, it is possible that the effect of these common co-morbid conditions could also have influenced the numbers of CECs in haemodialysed patients. Furthermore, the number of CECs after HD was significantly lower than pre-HD values, indicating a loss of CECs in the dialysis tubing and/or dialyzer. Thus, the HD procedure itself alters the level of CECs.

In another study, Koç et al. (2005) reported the incidence of vascular events in the cohort of HD patients from the first study (N = 27) after a follow-up of approximately 20 months. He divided the cohort of HD patients into two groups according to the number of CECs measured at the beginning of the study. All observed cardiovascular events (N = 5) and access-related events occurred in the "high-number" group, while no vascular event in the "low-number" group. Furthermore, in a second cohort of 44 conventional HD patients, CECs were correlated with markers of inflammation, namely high-sensitivity C-reactive protein, interleukin (IL)-6, IL-10, monocyte chemoattractant protein-1 (MCP-1), and vascular cellular adhesion protein-1 (VCAM-1). However, the authors did not find any significant correlation between these markers and CEC number. The authors conclude that in this cohort of conventional HD patients, an increase of CECs was found to predict cardiovascular and vascular (access-related) events, and to be independent of other markers of inflammation and endothelial dysfunction. This suggests that CECs could be a useful marker in predicting cardiovascular risk in conventional HD patients.

CECs in kidney transplantation

Several studies investigated the role of CECs in the field of kidney transplantation. In the 1970's Hladovec found that CEC counts were decreased in a group of patients after renal transplantation (Hladovec, 1976). His findings are in contrast with later studies, which show an increase of CECs in kidney transplant recipients. This discrepancy could be caused by the fact that cyclosporine – well-known cause of endothelial injury – was introduced in the transplantation medicine later, in the 1980's. A series of studies by Popa et al. (2002) and Woywodt et al. (2003b, c) showed that CEC numbers are elevated in patients after kidney transplantation and reflect the endothelial injury caused by allograft rejection or cytomegalovirus (CMV) infection. Most of the CECs present in the blood of kidney allograft recipients in the setting of acute rejection were of donor origin, thus reflecting vascular damage in the allograft (Popa et al., 2002). In the study by Woywodt (2003b), patients with acute vascular rejection had the highest cell numbers (72 ± 39 cells/ml) when compared with all other patients ($P < 0.02$). Regardless of their biopsy findings, however, all other renal transplant recipients had significantly higher numbers of CECs (25 ± 20 cells/ml) than healthy controls (7 ± 5 cells/ml, $P < 0.001$). Finally, there was a significant correlation of cell numbers and serum cyclosporine A trough levels. By contrast, there was no correlation with serum creatinine, age, or the number of immunosuppressive drugs. In another study by the same authors (Woywodt et al., 2003c), the hypothesis was tested that treatment with calcineurin inhibitors (i.e. cyclosporine, tacrolimus) leads to an increase in CECs. Fifty-seven renal transplant recipients were studied: 19 on a calcineurin inhibitor-free immunosuppressive regimen and 38 patients on a standard immunosuppressive regimen, including cyclosporine, and matched for age and serum creatinine. Patients with cyclosporine therapy had elevated numbers of circulating endothelial cells (median 26, range 12 to 82 cells/ml) compared to healthy controls (median 6, range 0 to 82 cells/ml; $P < 0.001$). Patients without calcineurin inhibitor treatment had significantly lower cell numbers (median 12, range 0 to 32 cells/ml; $P < 0.003$) and were not significantly different from normal, untreated controls. In conclusion, renal transplant recipients who do not receive calcineurin inhibitors have significantly lower numbers of CECs than

their age- and creatinine-matched counterparts who receive these drugs. The authors suggest that elevated numbers of CECs indicate damage from calcineurin inhibitors in renal transplant recipients and that CECs represent a novel marker of endothelial damage.

CECs and ANCA-associated vasculitis

In a study by Woywodt et al. (2003a), high numbers of cells (> 100) were detected in patients with active ANCA-associated systemic vasculitis (AAV) and cell numbers declined progressively during the course of successful immunosuppressive treatment. Moderately elevated numbers of CECs were detected in blood obtained from patients in remission. Finally, controls with infection and non-vasculitic renal disease did not have elevated cell numbers. Therefore, the positive predictive value of a cell count above 25 cells/ml was 100 %, while the negative predictive value was 97 %. The authors concluded that CECs are new markers of AAV, and may be markers of vasculitis in general. Interestingly, a necrotic phenotype as evidence of the severity of the inflammatory process and tissue-factor expression of the cells could be demonstrated. In a further study by the same group (Woywodt et al., 2006), CECs were measured in 16 patients with active AAV and 12 with limited granulomatous disease. Patients with vasculitic relapse had markedly increased numbers of CECs (12–800 cells/ml, median 88 cells/ml), as did patients with newly diagnosed systemic vasculitis (20–216 cells/ml, median 56 cells/ml). Patients with limited granulomatous disease due to Wegener's granulomatosis (WG) had only slightly increased cell numbers (4–44 cells/ml, median 20 cells/ml), which were similar to those of patients in remission (4–36 cells/ml, median 16 cells/ml). Numbers of CECs in patients with granulomatous disease were significantly lower than in those patients with relapse or new onset vasculitis ($P < 0.001$). Cell numbers in patients with relapse and new onset vasculitis declined with immunosuppressive treatment. Patients with infection had 4–36 cells/ml (median 10 cells/ml). A cut-off value of 20 cells/ml for a positive result yielded 64 % specificity and 95 % sensitivity for active systemic vasculitis; the positive predictive value was 63 % and the negative predictive value 95 %. In this study, markedly increased numbers of CECs were able to discriminate active vasculitis from limited granulomatous disease and remission.

Similar results were obtained by Holmén et al. (2005), who investigated a cell population called "circulating inflammatory endothelial cells (IECs)" defined as endothelial cells that express two inflammatory-associated markers: vascular adhesion protein-1 (VAP-1) and MHC class I-related chain A (MICA). The authors show that these cells are in fact identical with the population of CECs. IECs were increased significantly in patients with active disease as compared with those in remission. IEC levels significantly correlated with the level of C-reactive protein and extent of organ involvement. Furthermore, IECs expressed high levels of inducible nitric oxide synthases (iNOS) and produced chemokines that are known to recruit and activate neutrophils, and significantly inhibited proliferation, migration, and endothelial nitric oxide synthase expression in EPCs. Thus, apart from being a disease activity marker, IECs may directly contribute to vascular damage and hamper the mechanisms of vascular repair.

EPCs in renal disease – just biomarkers, or more?

In the past, regeneration of the injured endothelium has been attributed to the migration and proliferation of the neighbouring endothelial cells. More recent studies, however, indicate that additional repair mechanisms help to restore endothelial integrity. In the early studies (Brieler et al., 1982, Rafii et al. 1995), implanted Dacron grafts and the surface of ventricular assist devices were shown to be rapidly covered by bone marrow-derived cells. Asahara et al. (1997) were the first to describe cells circulating in peripheral blood that expressed markers of haematopoietic stem cells and were able to differentiate *in vitro* into endothelial cells. These cells seemed to possess the capacity of embryonic haemangioblasts and were called circulating endothelial progenitor cells (EPCs).

EPCs are characterized either immunologically (by expression of cell surface antigens) or by their ability to proliferate, adhere, and migrate in cell culture assays. Their definition is still evolving and it seems that the mononuclear fraction of peripheral blood harbours more cell types able to differentiate into the endothelial phenotype and support endothelial regeneration either by direct incorporation into the vessel wall or by paracrine effect. Various investigators define EPCs by co-expression of certain endothelial specific markers (such as VEGFR-2/ KDR, CD31, CD 146 and von Willebrand factor and progenitor/stem cell markers such as CD34 and CD133). An alternative is to evaluate their capacity to form colonies (colony-forming units) *in vitro* and to incorporate acetylated low-density lipoprotein and bind lectins.

In contrast to CEC data, where high numbers prevail, EPC counts are mostly reported to be lowered and inversely correlated with risk factors for and severity of vascular disease.

EPCs and end-stage kidney disease

End-stage renal disease (ESRD) is associated with a high prevalence of cardiovascular complications (Foley et al., 2005). ESRD patients suffer from a chronic inflammatory process, which causes endothelial injury (Pawlak et al., 2004), a critical event in the pathogenesis of atherosclerosis. Endothelial progenitor cells (EPCs) may play a key role in the endothelial homeostasis, and several research groups have investigated the numbers and function of EPCs in ESRD patients. The role of EPCs in ESRD was extensively reviewed elsewhere (Herbig et al., 2006b).

Eizawa et al. (2003) was the first who extensively studied EPCs in HD patients. He compared 50 HD patients and 36 healthy volunteers, measuring EPCs by flow cytometry ($AC133^+$ cells and $CD34^+$ cells) and by *in vitro* culture assay together with the levels of serum VEGF. The numbers of $CD34^+$ mononuclear cells and $AC133^+$ mononuclear cells were significantly reduced by 56% and 49%, respectively, in HD patients compared with control subjects. The number of EPCs determined by the culture assay was also significantly reduced by 41% in HD patients compared with control subjects. The serum VEGF levels in HD patients were not different from those in control subjects and did not correlate with EPC counts.

Similar results were reported by Choi et al. (2004). Again, EPCs were isolated from ESRD patients on maintenance HD (N = 44) and from a normal control group (N = 30). Patients with chronic renal failure showed markedly decreased numbers of EPCs (44.6%) and colonies (75.3%) when compared with the controls. These findings were corroborated by 30.5% decrease in EPC migratory function in response to VEGF and 48.8% decrease in EPC incorporation into human umbilical vein endothelial cells (HUVECs). In addition, Framingham's risk factor score of both ESRD and normal group significantly correlated with the numbers of EPCs. A significant correlation was also observed between dialysis dose (Kt/V) and ECP incorporation into HUVECs.

Others have supported the role of uraemia and dialysis dose for EPC physiology. De Groot et al. (2004) showed that uraemic serum significantly inhibited EPC differentiation and functional activity *in vitro*. Amelioration of uraemia after institution of renal replacement therapy in patients with terminal renal failure also significantly increased the number of EPCs. Chan et al. (2005) conducted a cross-sectional study in three cohorts: patients on conventional dialysis, nocturnal daily dialysis and healthy volunteers. Compared with control patients, patients on conventional HD exhibited an approximately 4-fold reduction in EPC number and function. Augmentation of uraemic clearance using nocturnal daily HD was associated with restored EPC number and migratory function. Furthermore, in ESRD patients, the EPC number was related to predialysis urea concentration, systolic blood pressure, and left ventricular mass index. In this fashion, this study identifies a novel potential mechanistic link between uraemic toxin burden, EPC number and function, and important surrogate outcomes, such as left ventricular mass index (LVMI), and underscores the possible importance of aggressive uraemic clearance, using e.g. nocturnal daily dialysis, for EPC survival and function.

Westerweel et al. (2007) studied the numbers of EPCs and smooth muscle progenitor cells (SPCs) in HD patients and the growth of these cells in the medium with added serum of patients with ESRD and healthy controls. Smooth muscle progenitors are able to differentiate into vascular smooth muscle cells and thus, contrary to EPCs, contribute to atherosclerotic plaque formation. This study has shown that in 45 ESRD patients receiving regular HD treatment, there were significantly reduced numbers of EPCs and low yield of EPCs cultured with uraemic serum. Interestingly, a dialysis session did not increase but markedly reduced circulating progenitor cells in the circulation – possibly because of sequestration or increased apoptosis of EPCs. In contrast, SPC numbers in ESRD patients were normal and SPC outgrowth in uraemic serum was unaffected. The authors conclude that the imbalance between EPCs and SPCs could contribute to the acceleration of cardiovascular disease in ESRD patients.

EPCs and kidney transplantation

Several studies investigated EPCs in renal transplant recipients (RTs). Soler et al. (2005) studied 94 RTs and 39 healthy controls. All RTs were treated by calcineurin inhibitors. EPCs (defined as $CD34^+$, $CD133^+$) were determined by flow cytometry. The authors have found that the EPC number was reduced compared to controls, especially in patients with reduced glomerular filtration rate (GFR). On the other hand, de Groot et al. (2005) found in a study on 74 RTs that EPC numbers in stable renal transplant recipients were comparable with healthy controls, while patients with pre-terminal renal failure (without renal replacement therapy or renal transplantation) had significantly lower numbers of EPCs. In a cross-sectional study in 105 middle-aged RTs by Steiner et al. (2006), no evidence of independent associations between several important cardiovascular risk factors (hyperlipidaemia, hyperglycaemia, smoking and inflammation) and EPC counts was found. Similarly, overall graft function was not associated with EPC counts, either. By contrast, greater BMI, higher mean blood pressure, and history of cardiovascular disease were associated with lower EPC counts in this RT population. Interestingly, in a follow-up study of 20 HD patients who subsequently underwent kidney transplantation, Herbig et al (2006a) found improvement in function of EPCs (assessed by the ability to migrate and adhere to fibronectin), but not in the number of EPCs (which was in fact lower).

EPCs and vasculitis

In a study by Holmén et al. (2005) low numbers of EPCs have been found in patients with active AAV. Furthermore, *in vitro*, inflammatory endothelial cells (IECs) produced soluble factors, which had a significant negative effect on the proliferation, migration and endothelial nitric oxide synthases (eNOS) expression of EPCs. Our own unpublished data support the low yield of EPC colonies from the blood of patients with AAV.

Involvement of EPCs in the kidney repair

The topic of uttermost importance for a nephrologist is the progression of chronic kidney disease, and halting of this process could be easily likened to the nephrologists' Holy Grail. The classical paradigm for the patho-

genesis of progressive renal disease is based on the assumption that the kidney scarring process after triggering renal injury is driven mainly by the homeostatic mechanisms involved in maintaining GFR in the setting of reduced nephron number. In response to a fall in GFR, there is stimulation of cyclooxygenase 2 in the macula densa, which leads, via stimulation of prostaglandins and renin, to dilatation of afferent arteriole and constriction of efferent arteriole, thus increasing systemic and glomerular hydrostatic pressure. This process causes endothelial and mesangial injury and induces proteinuria with resultant tubular and interstitial injury. The injured cells then stimulate local inflammatory and fibroproliferative response with ultimate apoptosis of the involved cells and fibrosis of the kidney tissue. However, an important component of the renal disease progression (not embedded in the above paradigm) is the role of ischemia. Ischemia could occur via intrarenal vasoconstriction or via arteriolar, glomerular, or peritubular capillary lesions that impair blood flow to the tubules. The maintenance of the microvasculature could thus be of critical importance, because viable endothelium is essential for the survival of other cells (e.g. mesangial, tubular) by virtue of its role in oxygen and nutrient delivery. The kidney harbours several different types of endothelial cells, in particular the endothelium of the macrovasculature, the peritubular capillary endothelium, and the glomerular endothelium. Besides participation in the filtration, re-absorption, and nutrition of the renal tissue, endothelial cells play a key role in recovery from several renal diseases. The evident question therefore is whether EPCs can also participate in regeneration and maintenance of these renal endothelial cell types. Several studies (Ikarashi et al., 2005, Uchimura et al., 2005, Li et al., 2006) have provided evidence that circulating EPCs may contribute to glomerular capillary repair – these investigators carried out similar experiments showing a protective role of bone marrow cell infusion in renal arteries of mice subjected to experimentally induced renal damage. Experiments with rat haematopoietic chimeras (Rookmaaker et al., 2007) have suggested that EPCs contribute to normal physiologic glomerular endothelial cell turnover. Following glomerular injury a 4-fold increase in bone marrow-derived endothelial cells in the glomeruli has been observed. These data indicate that glomerular repair can not only be attributed to migration and proliferation of resident endothelial cells, but also involves bone marrow-derived cells. In a rat animal model, Patschan et al. (2007) showed that renal ischemia rapidly mobilizes EPCs, which transiently home to the spleen, acting as a reservoir of mobilized EPCs. After ischemic preconditioning (in the late phase after several days) accumulation of EPCs in the renal medullopapillary region occurred. Transplantation of EPC-enriched cells from the medullopapillary parenchyma afforded partial renoprotection after renal ischemia, suggesting the role of the recruited EPCs in the functional rescue. Bussolati et al. (2005) reported that *in vitro* expanded renal-derived $CD133^+$ cells home to injured, but not into normal kidneys. These cells were capable of expansion, responded to local instructive cues, and were able to differentiate *in vitro* endothelial cells and to generate *in vivo* functional vessels. Although these cells were not typical EPCs but rather renal-derived stem cells, this observation supports the role of vasculogenesis as a contributor to the repair of vascular injury and in antagonizing the endothelial loss and progression to renal failure. Participation of circulating EPCs to renal regeneration has also been repeatedly demonstrated in human adults by studies of endothelial chimerism in renal allografts (Williams et al., 1969, Bai et al., 2007). Lagaaij et al. (2001) also reported that in human renal transplants the extent of replacement of donor endothelial cells lining the peritubular capillaries by those of the acceptor was related to the severity of vascular injury. Recently, Rookmaker et al. (2002) demonstrated male, donor-derived endothelial cells in the renal macrovasculature of a female patient who developed thrombotic microangiopathy after gender-mismatched bone marrow transplantation. Taken together, these observations confirm an important role of EPCs in the maintenance and repair of renal endothelium.

Conclusion

An emerging body of literature has determined that circulating EPCs maintain vascular integrity and are functional in the repair of damaged tissues. Their counterparts – CECs – could serve as markers of vascular injury or activity of certain disease processes (e.g. vasculitis) and predictors of cardiovascular outcomes. EPCs correlate inversely with cardiovascular risk factors and are involved in the repair of the damaged endothelium. Measures that improve the numbers of EPCs or their function (such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor antagonists, statins, erythropoietin, increased dialysis dose) could possibly improve the unfavourable cardiovascular risk profile of patients with chronic kidney disease. Furthermore, EPCs are recruited into the kidney in the healing phase of renal inflammation, and a deficiency in this process can result in persistent cell death and development of chronic disease. Augmentation of EPC-dependent repair by provision of EPC-stimulating chemokines or by direct transfer of EPCs into the kidney can enhance healing and limit the ongoing disease. Finally, it seems that EPCs are not just passive witnesses or victims of endothelial and tissue injury, but also important players (accomplices) in the field of endothelial homeostasis.

References

Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G., Isner, J. M. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* **275,** 964-967.

Bai, H. W., Shi, B. Y., Qian, Y. Y., Na, Y. Q., Zeng, X., Zhong, D. R., Lu, M., Zou, W. Z., Wu, S. F. (2007) Endothelial cell chimerism by fluorescence in situ hybridization in gender mismatched renal allograft biopsies. *Chin. Med. J. (Engl)* **120**, 859-862.

Basile, D. P. (2007) The endothelial cell in ischemic acute kidney injury: implications for acute and chronic function. *Kidney Int*. **72**, 151-156.

Boos, C. J., Soor, S. K., Kang, D., Lip, G. Y. (2007) Relationship between circulating endothelial cells and the predicted risk of cardiovascular events in acute coronary syndromes. *Eur. Heart J*. **28**, 1092-1101.

Brieler, H. S., Thiede, A., Beck, C. (1982) Monocytogenic endothelialization in dacron grafts. Experimental studies on rats. *J. Cardiovasc. Surg. (Torino)* **23**, 483-489.

Bussolati, B., Bruno, S., Grange, C., Buttiglieri, S., Deregibus, M. C., Cantino, D., Camussi, G. (2005) Isolation of renal progenitor cells from adult human kidney. *Am. J. Pathol.* **166**, 545-555.

Chan, C. T., Li, S. H., Verma S. (2005) Nocturnal hemodialysis is associated with restoration of impaired endothelial progenitor cell biology in end-stage renal disease. *Am. J. Physiol. Renal Physiol.* **289**, F679-684.

Choi, J. H., Kim, K. L., Huh, W., Kim, B., Byun, J., Suh, W., Sung, J., Jeon, E. S., Oh, H. Y., Kim, D. K. (2004) Decreased number and impaired angiogenic function of endothelial progenitor cells in patients with chronic renal failure. *Arterioscler. Thromb. Vasc. Biol.* **24**, 1246-1252.

Clancy, R., Marder, G., Martin, V., Belmont, H. M., Abramson, S. B., Buyon, J. (2001) Circulating activated endothelial cells in systemic lupus erythematosus: further evidence for diffuse vasculopathy. *Arthritis Rheum.* **44**, 1203-1208.

Cross, J. (2002) Endothelial dysfunction in uraemia. *Blood Purif.* **20**, 459-461.

de Groot, K., Bahlmann, F. H., Sowa, J., Koenig, J., Menne, J., Haller, H., Fliser, D. (2004) Uremia causes endothelial progenitor cell deficiency. *Kidney Int.* **66**, 641-646.

de Groot, K., Bahlmann, F. H., Bahlmann, E., Menne, J., Haller, H., Fliser, D. (2005) Kidney graft function determines endothelial progenitor cell number in renal transplant recipients. *Transplantation* **79**, 941-945.

Del Papa, N., Colombo, G., Fracchiolla, N., Moronetti, L. M., Ingegnoli, F., Maglione, W., Comina, D. P., Vitali, C., Fantini, F., Cortelezzi, A. (2004) Circulating endothelial cells as a marker of ongoing vascular disease in systemic sclerosis. *Arthritis Rheum.* **50,** 1296-1304.

Eizawa, T., Murakami, Y., Matsui, K., Takahashi, M., Muroi, K., Amemiya, M., Takano, R., Kusano, E., Shimada, K., Ikeda, U. (2003) Circulating endothelial progenitor cells are reduced in hemodialysis patients. *Curr. Med. Res. Opin.* **19**, 627-633.

Foley, R. N., Murray, A. M., Li, S., Herzog, C. A., McBean, A. M., Eggers, P. W., Collins, A. J. (2005) Chronic kidney disease and the risk for cardiovascular disease, renal replacement, and death in the United States Medicare population, 1998 to 1999. *J. Am. Soc. Nephrol.* **16**, 489-495.

George, F., Brouqui, P., Boffa, M. C., Mutin, M., Drancourt, M., Brisson, C., Raoult, D., Sampol, J. (1993) Demonstration of Rickettsia conorii-induced endothelial injury in vivo by measuring circulating endothelial cells, thrombomodulin, and von Willebrand factor in patients with Mediterranean spotted fever. *Blood* **82,** 2109-2116.

Herbrig, K., Gebler, K., Oelschlaegel, U., Pistrosch, F., Foerster, S., Wagner, A., Gross, P., Passauer, J. (2006) Kidney transplantation substantially improves endothelial progenitor cell dysfunction in patients with end-stage renal disease. *Am. J. Transplant.* **6**, 2922-2928.

Herbrig, K., Pistrosch, F., Foerster, S., Gross, P. (2006). Endothelial progenitor cells in chronic renal insufficiency. *Kidney Blood Press Res.* **29,** 24-31.

Hladovec, J., Jirka, J., Prerovsky, I., Malek, P. (1976) Decrease of endothelaemia during immunosuppression. *Biomedicine* **6**, 204-206.

Holmén, C., Elsheikh, E., Stenvinkel, P., Qureshi, A. R., Pettersson, E., Jalkanen, S., Sumitran-Holgersson, S. (2005) Circulating inflammatory endothelial cells contribute to endothelial progenitor cell dysfunction in patients with vasculitis and kidney involvement. *J. Am. Soc. Nephrol.* **16**, 3110-3120.

Ingram, D. A., Caplice, N. M., Yoder, M. C. (2005) Unresolved questions, changing definitions, and novel paradigms for defining endothelial progenitor cells. *Blood* **106**, 1525-1531.

Ikarashi, K., Li, B., Suwa, M., Kawamura, K., Morioka, T., Yao, J., Khan, F., Uchiyama, M., Oite, T. (2005) Bone marrow cells contribute to regeneration of damaged glomerular endothelial cells. *Kidney Int.* **67,** 1925-1933.

Kang, D. H., Kanellis, J., Hugo, C., Truong, L., Anderson, S., Kerjaschki, D., Schreiner, G. F., Johnson, R. J. (2002) Role of the microvascular endothelium in progressive renal disease. *J. Am. Soc. Nephrol.* **13**, 806-816.

Koc, M., Bihorac, A., Segal, M. S. (2003) Circulating endothelial cells as potential markers of the state of the endothelium in hemodialysis patients. *Am. J. Kidney Dis.* **42**, 704-712.

Koc, M., Richards, H. B., Bihorac, A., Ross, E. A., Schold, J. D., Segal, M. S. (2005) Circulating endothelial cells are associated with future vascular events in hemodialysis patients. *Kidney Int.* **67**, 1078-1083.

Lagaaij, E. L., Cramer-Knijnenburg, G. F., van Kemenade, F. J., van Es, L. A., Bruijn, J. A., van Krieken, J. H. (2001) Endothelial cell chimerism after renal transplantation and vascular rejection. *Lancet* **357**, 33-37.

Li, B., Morioka, T., Uchiyama, M., Oite, T. (2006) Bone marrow cell infusion ameliorates progressive glomerulosclerosis in an experimental rat model. *Kidney Int.* **69,** 323-330.

Makin, A. J., Blann, A. D., Chung, N. A., Silverman, S. H., Lip, G. Y. (2004) Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells. Relationship with von Willebrand factor and tissue factor. *Eur. Heart J.* **25**, 371-376.

Mutin, M., Canavy, I., Blann, A., Bory, M., Sampol, J., Dignat-George, F. (1999) Direct evidence of endothelial injury in acute myocardial infarction and unstable angina by demonstration of circulating endothelial cells. *Blood* **93,** 2951-2958.

Ohashi, R., Kitamura, H., Yamanaka, N. (2000) Peritubular capillary injury during the progression of experimental glomerulonephritis in rats. *J. Am. Soc. Nephrol.* **11**, 47-56.

Ojo, A. O. (2006) Cardiovascular complications after renal transplantation and their prevention. *Transplantation* **82**, 603-611.

Patschan, D., Krupincza, K., Patschan, S., Zhang, Z., Hamby, C., Goligorsky, M. S. (2006) Dynamics of mobilization and homing of endothelial progenitor cells after acute renal ischemia: modulation by ischemic preconditioning. *Am. J. Physiol. Renal Physiol.* **291,** F176-185.

Patschan, D., Michurina, T., Shi, H. K., Dolff, S., Brodsky, S. V., Vasilieva, T., Cohen-Gould, L., Winaver, J., Chander, P. N., Enikolopov, G., Goligorsky, M. S. (2007) Normal distribution and medullary-to-cortical shift of nestin-expressing cells in acute renal ischemia. *Kidney Int.* **71**, 744-754.

Pawlak, K., Naumnik, B., Brzósko, S., Pawlak, D., Myśliwiec, M. (2004) Oxidative stress - a link between endothelial injury, coagulation activation, and atherosclerosis in haemodialysis patients. *Am. J. Nephrol.* **24,** 154-161.

Popa, E. R., Kas-Deelen, A. M., Hepkema, B. G., Van Son, W. J., The, T. H., Harmsen, M. C. (2002) Donor-derived circulating endothelial cells after kidney transplantation. *Transplantation* **74,** 1320-1327.

Rafii, S., Oz, M. C., Seldomridge, J. A., Ferris, B., Asch, A. S., Nachman, R. L., Shapiro, F., Rose, E. A., Levin, H. R. (1995) Characterization of hematopoietic cells arising on the textured surface of left ventricular assist devices. *Ann. Thorac. Surg.* **60,** 1627-3162.

Rookmaaker, M. B., Tolboom, H., Goldschmeding, R., Zwaginga, J. J., Rabelink, T. J., Verhaar, M. C. (2002) Bone-marrow-derived cells contribute to endothelial repair after thrombotic microangiopathy. *Blood* **99**, 1095.

Rookmaaker, M. B., Smits, A. M., Tolboom, H., Van, T., Wout, K., Martens, A. C., Goldschmeding, R., Joles, J. A., Van Zonneveld, A. J., Gröne, H. J., Rabelink, T. J., Verhaar, M. C. (2003) Bone-marrow-derived cells contribute to glomerular endothelial repair in experimental glomerulonephritis. *Am. J. Pathol.* **163**, 553-562.

Rookmaaker, M.B., Verhaar, M. C., van Zonneveld, A. J., Rabelink, T. J. (2004) Progenitor cells in the kidney: biology and therapeutic perspectives. *Kidney Int.* **66**, 518-522.

Rookmaaker, M. B., Verhaar, M. C., de Boer, H. C., Goldschmeding, R., Joles, J. A., Koomans, H. A., Grone, H. J., Rabelink, T. J. (2007) Met-RANTES reduces endothelial progenitor cell homing to activated (glomerular) endothelium in vitro and *in vivo*. *Am. J. Physiol. Renal Physiol.* **293**, F624-630.

Quilici, J., Banzet, N., Paule, P., Meynard, J. B., Mutin, M., Bonnet, J. L., Ambrosi, P., Sampol, J., Dignat-George, F. (2004) Circulating endothelial cell count as a diagnostic marker for non-ST-elevation acute coronary syndromes. *Circulation* **110,** 1586-1591.

Segal, M. S., Bihorac, A., Koc, M. (2002) Circulating endothelial cells: tea leaves for renal disease. *Am. J. Physiol. Renal Physiol* .**283,** F11-19.

Steiner, S., Winkelmayer, W. C., Kleinert, J., Grisar, J., Seidinger, D., Kopp, C. W., Watschinger, B., Minar, E., Hörl, W. H., Födinger, M., Sunder-Plassmann, G. (2006) Endothelial progenitor cells in kidney transplant recipients. *Transplantation* **27**, 599-606.

Soler, M. J., Martínez-Estrada, O. M., Puig-Marí, J. M., Marco-Feliu, D., Oliveras, A., Vila, J., Mir, M., Orfila, A., Vilaró, S., Lloveras, J. (2005) Circulating endothelial progenitor cells after kidney transplantation. *Am. J. Transplant.* **5**, 2154-2159.

Uchimura, H., Marumo, T., Takase, O., Kawachi, H., Shimizu, F., Hayashi, M., Saruta, T., Hishikawa, K., Fujita, T. (2005) Intrarenal injection of bone marrow-derived angiogenic cells reduces endothelial injury and mesangial cell activation in experimental glomerulonephritis. *J. Am. Soc. Nephrol*. **16,** 997-1004.

Westerweel, P. E., Hoefer, I. E., Blankestijn, P. J., de Bree, P., Groeneveld, D., van Oostrom, O., Braam, B., Koomans, H. A., Verhaar, M. C. (2007) End-stage renal disease causes an imbalance between endothelial and smooth muscle progenitor cells. *Am. J. Physiol. Renal Physiol.* **292**, F1132-1140.

Williams, G. M., Alvarez, C. A. (1969) Host repopulation of the endothelium in allografts of kidneys and aorta. *Surg. Forum* **20**, 293-294.

Woywodt, A., Schroeder, M., Gwinner, W., Mengel, M., Jaeger, M., Schwarz, A., Haller, H., Haubitz, M. (2003) Elevated numbers of circulating endothelial cells in renal transplant recipients. *Transplantation* **76**, 1-4.

Woywodt, A., Streiber, F., de Groot, K., Regelsberger, H., Haller, H., Haubitz, M. (2003) Circulating endothelial cells as markers for ANCA-associated small-vessel vasculitis. *Lancet* **361**, 206-210.

Woywodt, A., Schroeder, M., Mengel, M., Schwarz, A., Gwinner, W., Haller, H., Haubitz, M. (2003) Circulating endothelial cells are a novel marker of cyclosporine-induced endothelial damage. *Hypertension* **41**, 720-723.

Woywodt, A., Goldberg, C., Kirsch, T., de Groot, K., Haubitz, M. (2006) Circulating endothelial cells in relapse and limited granulomatous disease due to ANCA associated vasculitis. *Ann. Rheum. Dis.* **65**, 164-168.