Circulating endothelial cells in renal disease: markers and mediators of vascular damage

Alexander Woywodt1, Torsten Kirsch2 and Marion Haubitz2

1Renal Unit, Lancashire Teaching Hospitals NHS Foundation Trust, Preston, Lancashire, UK and 2Division of Nephrology, Department of Medicine, Hannover Medical School, Hannover, Germany

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Introduction

During the last decade, circulating endothelial cells (CECs) have been used as a surrogate marker of endothelial damage in a variety of vascular disorders. The severely damaged phenotype of CECs in vasculitis led to the hypothesis that such circulating apoptotic and/or necrotic debris may itself be a mediator of disease. Very recently, first evidence has emerged to support this assumption [1]. The aim of this editorial comment is to provide a brief review of CECs, describe clinical applications with an emphasis on renal disease, and put the new pathogenetic findings into perspective. We also suggest further avenues of research.

CECs: promise and problems

The concept of circulating endothelial cells is not new. These cells were first described almost 40 years ago [2], although identification of these cells by light microscopy was rather primitive from today’s point of view. Some studies on cardiovascular diseases followed until the next milestone in methodology, the discovery of the S-Endo 1 antigen [3], was reached. The respective antibody then permitted immunomagnetic isolation as a more elegant technique [2]. More recently, fluorescence-activated cell sorting (FACS) has been employed, and both techniques have their advantages and disadvantages [4]. A particular strength of immunomagnetic isolation is the use of morphological criteria, but the technique needs attention to technical detail [5,6]. At first glance, multi-parametric FACS [7] appears more user-friendly, not least because leukocyte contamination can be excluded with careful use of markers and gating. However, the technique does not permit direct observation of the cell morphology, and it needs no less attention to technical detail than immunomagnetic isolation. A detailed comparison of the two competing approaches can be found elsewhere [4]. There is an ongoing debate as to which of the two may be preferable, while the entire field is still markedly hampered by the fact that there is no uniformly accepted set of surface markers for CECs. Consensus and standardization are very important when the clinical use of CECs is contemplated, and overlap between mature CECs and bone-marrow derived endothelial progenitor cells (EPCs) is a matter of particular concern [8]. Both a definition for CECs and a consensus methodology for their isolation have been proposed in a recent European multi-centre effort [9].

CECs in renal disease

Vasculitis associated with anti-neutrophil cytoplasmic antibodies (ANCA) serves as a paradigm of an endothelial disorder, and CECs are a valuable marker of disease, as reviewed in great detail elsewhere [5,10,11]; we have found CECs very useful to monitor treatment and to distinguish between relapse and infection in difficult cases, but the clinical utility of this marker needs to be corroborated by others. Interestingly, recent data suggest an increase in EPCs in vasculitis patients after induction of immunosuppressive treatment, suggesting ongoing repair mechanisms [12]. It is clear that our findings are not specific to the ANCA-associated variant of vasculitis: Dang et al. reported elevated CEC numbers in aortoarteritis [13], while Nakatani et al. demonstrated CECs in patients with Kawasaki disease [14]. Thrombotic microangiopathy (TMA) is another micro-vascular disorder, and patients with a favourable outcome have significantly higher initial CEC levels and a decrease in cell numbers with successful plasma exchange [15]. A vexing variant of TMA is that associated with haematopoietic stem cell transplantation (HSCT): along with other poorly understood endothelial complications of HSCT, such as veno-occlusive disease of the liver, HSCT-associated TMA is a therapeutic challenge. We studied the CEC numbers during the conditioning phase of HSCT and observed a marked rise in CEC...
numbers as well as a correlation with the conditioning ‘dose’ [16]. It would be interesting to study whether early rises in CEC numbers precede and indeed predict vascular complications [17]. Data in other glomerular disorders are very limited. Futtrakul et al. demonstrated elevated CEC numbers in Focal segmental glomerulosclerosis (FSGS) [18], although it is difficult to understand how endothelial damage may develop in a podocyte disorder. Other factors, such as the effects of treatment, may be at play. We did not find elevated CECs in only a very limited number of GN patients [5].

Renal transplantation is another interesting field to study and CECs may reflect vascular rejection [19], the effects of calcineurine inhibitors [20] or both. The cells appear to be of donor origin [21], a finding that supports previous reports of endothelial chimerism in renal grafts [22]. Infection in the immunocompromised host is also worth investigating: cytomegalovirus, for example, is an endothelial pathogen and the virus is detectable in circulating endothelial giant cells [23]. Other herpes viruses, such as human herpes virus 8, have not been studied so far, not least because the methodology of such studies is sophisticated.

Finally, chronic renal failure has been studied, although CECs work best with acute, widespread, endothelial damage as in vasculitis; hence, data are less impressive than in vasculitis or TMA. Elevated numbers of CECs were described in haemodialysis patients [24]. A subsequent study reported that elevated CEC numbers convey a risk of future vascular events [25]. Patients with end-stage renal failure due to diabetic nephropathy have not been studied in any great detail, although it is known that CECs are elevated in type II diabetes [26]. Surprisingly, a comparison between haemodialysis and peritoneal dialysis has not been done, and it would be interesting to study patients who change from haemodialysis to peritoneal dialysis, or vice versa. Finally, a very recent study postulates an imbalance between CECs and EPCs in patients with chronic renal failure [27]. Along with similar studies in vasculitis [28], these findings are intriguing but need independent confirmation.

**Circulating endothelial cells as mediators of disease**

Little is known about the phenotype of circulating endothelial cells, but preliminary studies indicated a pro-coagulant phenotype [5]. Several lines of evidence suggest that circulating endothelial cells could be pro-inflammatory [29]. In general, damaged cells have been shown to release a variety of pro-inflammatory factors, initiate a Toll-like-receptor/2/NFκB-dependent reaction in monocytes and fibroblasts [30], or induce transforming growth factor beta (TGF-beta) in macrophages [31]. It was proposed that damaged CECs trigger similar effects [29], but such mechanisms have never been demonstrated. It was also believed that dying or dead cells are rapidly removed by professional macrophages or by neighbouring cells before their inflammatory potential can unfold. It was finally hypothesized that in disease, CECs may be present in high numbers, overwhelm the clearance mechanisms, initiate a series of signalling processes and thereby gain pathogenic importance [29].

Little is known about the fate of CECs in vivo, and mechanisms of detachment as well as CEC kinetics remain enigmatic. In addition, endothelial microparticles have been described but their relationship with CECs remains ill-defined. Of note, endothelial microparticles are also elevated in dialysis patients [32]. To make matters even more complicated, not all microparticles are endothelium-derived [33] and technical issues are similar to those of CECs. These particles are usually evaluated with flow cytometry, and several reports have proposed pathogenetic effects: it has been demonstrated that endothelial microparticles are tissue-factor positive [34], and very recent evidence suggests that they can also convert plasminogen into plasmin [35]. It is not known whether CECs and microparticles reflect the same disease process; a direct comparison between the two in the same patient would thus be of interest, while there is probably no great difference in expenditure and cost.

Evidence has emerged that ANCA accelerates apoptosis in neutrophils, and impaired clearance of apoptotic neutrophils has been described [36]. As a result, the healthy endothelium may encounter a vast array of apoptotic and/or necrotic cells and their debris. Interestingly, disturbed clearance of apoptotic cells has also been implicated in systemic lupus erythematosus [37]. It has been postulated recently that CECs may impair EPCs in vasculitis patients [28]. These findings need to be corroborated by further studies because such impairment cannot be a universal principle—otherwise, vascular lesions could never heal. In summary, several lines of evidence suggested that interactions enable healthy endothelium to sense ongoing apoptosis and necrosis in its vicinity, although such an assumption [29] has remained unproven for many years.

To prove interactions between CECs and healthy endothelium, the first challenge was to establish a suitable *in vitro* model. There were major obstacles to this endeavour, not least the fact that cultured endothelial cells from various tissues and vessel sizes are available. These include human umbilical vein and artery endothelial cells (HUVEC and HUAEC, respectively) as well as human micro-vascular endothelial cells (HMEC). The technique to induce apoptosis and necrosis in endothelial cells is also far from trivial. Chemical inducers may cause untoward effects because the treated apoptotic/necrotic cells may retain traces of the substance, release them into the supernatant and thus affect the healthy endothelial cell layer. In summary, ultraviolet (UV) light seemed to be the best option.

Most importantly, we were able to demonstrate that apoptotic and necrotic cells and their fragments are rapidly internalized by healthy endothelium (Figure 1). Confocal microscopy confirmed these findings and gave us further confidence in the interpretation. Support for these findings came from other studies demonstrating the phagocytic capability of endothelial cells [38]. We could also show that endothelial cells exposed to apoptotic and necrotic cells exhibit enhanced adhesion properties for leukocytes and that isolated CECs from patients with vasculitis even
aggravated these effects [1]. These effects on binding properties could be explained, at least in part, by the release of the pro-inflammatory chemo-attractants IL-8 and MCP1. Interestingly, apoptotic and necrotic cells induced different patterns of effects in healthy endothelium. Enhanced IL-8 and MCP1 levels in serum have been detected in patients with active vasculitis, and ANCA induces the synthesis of these chemokines in various cell subsets [39]. Endothelial synthesis of these mediators triggered by ANCA [40] and circulating endothelial cells [1] may contribute to the pro-inflammatory state associated with vasculitis.

What are the clinical implications of our findings? The mechanisms described here may limit and/or amplify disease: phagocytosis of CECs may remove the potentially dangerous debris from the circulation, while this event itself triggers pro-inflammatory pathways. Preliminary data of our group indicate that prolonged exposure of healthy endothelium to apoptotic/necrotic endothelial cells leads to down-regulation of IL-8 and MCP1. These observations add further support to the concept of anti-inflammatory feedback loops. All these mechanisms may be even more complicated: numbers of CECs in vasculitis may differ markedly between, say, a micro-vascular environment and the superior cava. Cytokine profiles may differ as well, and these may further influence interactions between CECs and healthy endothelium.

**Conclusion**

Our results are a proof of principle for interactions between apoptotic/necrotic endothelial cells and healthy endothelium (Figure 2). It makes a lot of sense to think of this as a mechanism by which healthy endothelium is alerted to the ongoing endothelial cell death. Such effects may well contribute to a pro-inflammatory environment in vascular disease. These mechanisms and the respective signalling pathways certainly deserve further study. For now, many aspects of CECs, such as their phenotype and kinetics after detachment, remain enigmatic.

**Conflict of interest statement:** None declared.