Endothelial progenitor cells, endothelial cell dysfunction and much more: observations from cardiac syndrome X

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Endothelial progenitor cells (EPCs) are a recent addition to the expanding field of vascular biology research. Described in the seminal study by Asahara *et al*,¹ they are now recognised as being significant contributors to adult vasculogenesis, angiogenesis and repair, with most studies pointing to a bone marrow origin. EPCs mobilise in response to specific triggers such as ischaemia and infarction, and EPC numbers correlate negatively with cardiovascular risk factors and atherosclerosis,² making them a useful prognostic surrogate.

Despite the overwhelming evidence for their presence, the *defining* pathophysiological role of EPCs is still the subject of much discussion, with many pertinent questions remaining. For example, do EPCs contribute mostly by a “promoting” influence on local vasculature—rather than by actively integrating into existing endothelium—and do they play an active part in reversing existing endothelial dysfunction?³ Whatever their true role, it is unquestionable that EPCs represent an important putative progenitor cell population with likely implications for continued vascular biology research and therapeutic agents.

Endothelial damage/dysfunction is present in many cardiovascular disorders, and is intimately associated with the risk factors predisposing to coronary artery disease (CAD). As the presence of EPCs may reflect a “general state of endothelial health”,⁴ it is not too unsurprising that EPCs are further defined in yet another cardiac disease reflecting endothelial dysfunction.

In this issue of *Heart* (see article on page 1071), Shmilovich *et al*⁵ rightly seek to elucidate further the pathogenic factors behind the enigmatic cardiac syndrome X (CSX), a disease postulated to derive from coronary microvascular dysfunction—and the resulting *microvascular ischaemia*—by quantifying EPC populations and functionality in patients with CSX. Nonetheless, CSX is a rather frustrating syndrome for researchers and clinicians alike because of the vagaries in diagnosis, ineffectual treatments and an unresolved pathophysiological origin. The earlier philosophy of CSX treatment was largely influenced by a seemingly good prognosis in these patients, albeit with a less than satisfactory quality of life in most.

Given these latest observations on EPCs in CSX from Shmilovich *et al*,⁵ it is possible that CSX is a disease entity stemming from overt microvascular endothelial dysfunction, perhaps with an

inflammatory component.⁶ More recent reports strongly suggest that patients with CSX with *demonstrable* endothelial dysfunction are at much higher risk of cardiovascular adverse events, more likely to develop angiographic evidence of CAD and have an overall poorer prognosis.⁷ As the demonstration of ischaemia by stress testing is no longer essential for the diagnosis of CSX, perhaps confirmation of endothelial dysfunction in CSX may eventually be the diagnostic test for this enigmatic syndrome.⁷

Shmilovich *et al*⁵ report significant differences in EPC numbers (increased), and severe in vitro EPC dysfunction (reduced proliferative ability) in patients with CSX compared with the control group (matched for cardiovascular risk factors). This increase in EPC numbers in CSX is intriguing and worth a comment. We know that tissue trauma, ischaemia and more recently, inflammation⁸ are all stimuli for EPC mobilisation, with associated growth factors—such as vascular endothelial growth factor—being implicated. As has been suggested by Shmilovich *et al*,⁵ if patients with CSX really do exhibit continuing inflammation and “microvascular ischaemia”, undetectable by normal stress methods, might not this explain an apparent EPC increase? Second, the raised EPC levels might be viewed as a favourable finding (bearing in mind that CSX has a relatively good prognosis), amid the suggestions that EPCs have a protective role on the vascular network, besides their potent angiogenic effects.⁴

Possibly of more significance is their finding that EPCs in CSX are functionally *ineffective*, with a poor proliferative index. This suggests that the microvasculature in CSX is somehow dependent upon EPC function, and the resulting endothelial dysfunction is a reflection of their inability to succour already ailing endothelium. Taken together, this would imply that patients with CSX exhibit a responsive EPC rise to certain triggering factors but cannot effect any significant improvement in endothelial dysfunction, thus compounding the clinical scenario. As with most problems, there always seems to be another side to the coin, one which perhaps appears far more sinister.

The possibility that EPCs are themselves vehicles of disease has previously been raised and debated. Indeed, Shmilovich *et al*⁵ show that cultured medium from EPCs from patients with CSX

Abbreviations: CAD, coronary artery disease; CSX, cardiac syndrome X; EPC, endothelial progenitor cell; PBMCs, peripheral blood mononuclear cells

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retards the growth of normal endothelium. Despite the fact that they do not go on to determine the precise inhibiting humoral/cytokine mechanism(s) for this, it does raise the possibility that EPCs have a detrimental effect, mediating endothelial dysfunction in CSX. If confirmed, this again opens up potential avenues in our understanding of EPC function and effect. Indeed, therapeutic strategies to specifically engender EPC health might well be attempted. Also, the presence of EPCs in CSX might conceivably have a similar prognostic significance to that of the CAD setting,⁴ helping to distinguish patients with CSX and "more severe" endothelial dysfunction from the commoner, innocuous form.

Despite this interesting EPC study in CSX, there still remains the continuing controversy about EPC definitions, which merits comment. In their paper, Shmilovich *et al*⁵ define EPCs by flow cytometry (FACS) as well as culture; this dual-method approach is fast gaining acceptance among EPC researchers. By FACS, EPCs were defined as separate populations of either CD34+/KDR+ or CD34+/CD133+ cells, with haematopoietic progenitor cells defined separately as CD34+/CD45+. The differentiation of progenitor populations into "haematopoietic" and "non-haematopoietic" would seem rather convenient and practical, but may yet prove too simplistic. Recent evidence controversially suggests that EPCs are derived largely from monocytic/macrophage cell lines (ie, CD14+/CD45+),⁹ whereas others have demonstrated that endothelial cells derived from peripheral blood mononuclear cells (PBMCs) represent a *mixed* population—including both mature and immature endothelial cells, cells assuming endothelial phenotype derived from monocytic/macrophage lines, and stem cells.¹⁰ It is felt that among this rather heterogeneous population, "true EPCs" are in the extreme minority.

A second consideration concerns the widely held assumption that PBMC-derived endothelial cell colonies correspond to circulating levels of EPCs. Bearing in mind cultured EPC heterogeneity, the ideal experimental evidence required would involve sorting circulating EPCs by FACS, and culturing these cells (a difficult task given that they represent an extremely rare cell population). Also, growing PBMCs in vitro in optimal, non-physiological "endothelial-promoting" culture medium will cause cells to assume an endothelial phenotype, something not normally seen.⁹ Thus, much work is still required to clarify what are "true" and "assumed" EPCs, as well as their individual and collective roles in both disease and health.

Despite our current knowledge of CSX, many unanswered questions remain.¹¹ The recent introduction of EPCs as an experimental tool in vascular research has helped us understand various conditions—such as CSX—by suggesting that endothelial dysfunction may be a primary disease mechanism. Indeed, raised absolute levels of impaired EPCs themselves apparently do not help, as these might instead be detrimental if functionality is still impaired. Additionally, the pathophysiological sequelae—such as the links to the "vascular triad" of thrombogenesis, atherogenesis or angiogenesis¹²—require careful definition. Whatever their true course in CSX—and indeed, cardiovascular disease—clearly, the study of EPC vascular biology is here to stay.

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