EDITORIAL COMMENT

Endothelial Progenitors

A New Tower of Babel?*

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One of the most fascinating breakthroughs in the field of vascular biology in the last decade was the discovery of endothelial progenitor cells (EPCs) (1). These angiogenic cells, with properties of embryonal angioblasts, are bone marrow residents, and mobilize in response to various stimuli (2). Endothelial progenitor cells may contribute to the maintenance of the vasculature and to the remodeling that accompanies new vessel growth with ischemia. Reflecting their proposed sentinel function, the number of circulating EPCs has been suggested to mirror vascular health and to represent a marker by which to assess cardiovascular disease risk (3–6). However, despite extensive research activity, surprisingly, there is no accepted standard method or criteria for defining an EPC. The lack of a uniform EPC definition complicates cross-study comparisons and may contribute to the apparent paradox of some studies suggesting that EPCs are reduced in the presence of cardiovascular risk factors and coronary artery disease (CAD) (4,6–10), whereas others suggest that numbers are increased in those with obstructive CAD (11,12).

WHO’S THAT CELL? EPC NOMENCLATURE

In the hierarchy established in the hematopoietic system, progenitors identify cells with lower differentiation potential than stem cells. However, EPCs possess degrees of “stemness”, which include self-renewal, clonogenicity, and differentiation capacity (13,14). Circulating EPCs were initially identified through their expression of CD34 (a surface marker common to hematopoietic stem cells and mature endothelial cells) and vascular endothelial cell growth factor receptor 2 (VEGFR2 or kinase-domain-related [KDR] receptor), but not of other markers seen on fully differentiated endothelial cells (1). Subsequent studies have incor-

porated other markers, such as the stem-cell marker CD133, which is not expressed by more differentiated cells. Other methods of characterization are based on in vitro behavior, including the ability to form endothelial colonies—colony-forming units (CFUs)—with the incorporation of acetylated low-density lipoprotein and binding of lectins (6). However, endothelial progenitor cells defined in these ways probably represent a mixed population, which, in combination with the lack of a consensual definition, make the interpretation and comparison of works in this field impossible. The current chaotic situation was highlighted by George et al. (15), who found that in healthy individuals there was no correlation between the various methods used for estimating EPC numbers.

In vitro studies have suggested that at least 2 populations of EPCs exist. “Early EPCs” (4 days), also called circulating angiogenic cells (CACs), are monocyte derived, are capable of assuming endothelial features, and produce angiogenic cytokines (2). The “true” endothelial precursor cell population, capable of generating late outgrowth of endothelial cells, are rare within the circulation (<0.01%), appear later (14 to 21 days) in culture, and likely originate from a subset of CD14+/CD34+/KDR+ cells that are not fully defined (2). However, the exact phenotypic characterization still remains unclear, and definitions should be made with caution.

EPCS AND CAD: POSITIVE OR NEGATIVE RELATIONSHIP?

Several physiologic stimuli, disease states, and drugs have been shown to influence EPC number and function (2). Although growing evidence suggests that circulating EPCs are depleted and exhausted in the presence of atherosclerosis risk factors (7–10), reports on circulating EPC number and CAD fail to show agreement. Several investigators report levels to be lower in disease states, although some have found increased EPCs in more severe disease. Vasa et al. (16) reported lower numbers of EPCs in patients with CAD compared with healthy controls, and the numbers of EPCs correlated inversely with the number of risk factors. Adams et al. (17) also reported somewhat lower EPC numbers in patients with CAD than in healthy controls, whereas exercise resulted in a transient increase. George et al. (11) reported higher EPCs in patients with unstable, compared with stable, angina as well as a positive correlation between EPC colony-forming units and the inflammatory marker CRP. Others found increased EPCs in acute myocardial infarction and chronic stable angina compared with controls (18,19), as well as after coronary bypass surgery (7). Lambiase et al. (12) have shown that poor coronary collateral development is associated with reduced numbers of circulating EPCs. However, they also found that patients with high collateral flow indices had more severe coronary stenoses and likely more severe myocardial ischemia. It was thus difficult to be certain whether EPCs relate to collaterals.

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or ischemia. Moreover, circulating EPCs may contribute to progression of atherosclerosis, as shown in animal models and transplant atherosclerosis (20–22).

Despite their complexities, the popular concept is that circulating EPCs are protective, and their lack mirrors and predicts disease progression and future cardiovascular events. Very recent studies by Werner et al. (3) and Schmidt-Lucke et al. (5) have shown that reduced numbers of circulating CD34+/KDR+/ EPCs predict the occurrence of cardiovascular events and death.

**THE PRESENT STUDY RAISES MORE QUESTIONS**

In this issue of the Journal, Güven et al. (23) have attempted to carefully address the relationship between circulating EPCs and CAD. They used an in vitro assay adopted from Ingram et al. (14) to measure the number of EPCs and CACs grown from blood samples of 48 patients undergoing coronary angiography. Patients with acute coronary syndromes were excluded. Their definition of EPCs was cells that eventually formed colonies of mature endothelial cells after 14 to 28 days in culture. In contrast to several previous reports, the number of EPC and CAC colonies was increased in proportion with the severity of CAD. In addition, in the population studied, and in contrast to previous reports, the level of EPCs did not vary significantly with age, cardiovascular risk factors, or CRP level. Thus, the study raises more questions about the value of EPCs as a valid diagnostic and prognostic tool.

The methodology of the present study is different from many others. An in vitro assay was used and “true” EPCs were identified by the formation of discrete colonies of endothelial cells on days 14 to 28 of culture. Many previous reports assess colony number earlier (7 days) and/or measure surface antigen expression by flow activated cell sorter. The use of an in vitro assay in isolation may be affected by culture conditions such as efficiency of adhesion to plastic ware, proliferation, and survival in culture (15). Endothelial cells cultured from peripheral blood do not directly correspond to the actual population of circulating EPCs because they may include other circulating cells with an endothelial cell phenotype. Taken together, the results of the present study, although provocative, do not provide a definitive answer to the question: what is the relationship between EPCs and CAD? The uncertainty is the result of using diverse methodologies to identify EPCs.

**WE NEED A STANDARD BY WHICH TO DEFINE EPCs**

Variations in methodology have created confusion regarding the interpretation of EPC findings across studies. Considering the growing evidence in support of circulating EPCs as a marker (positive or negative) of vascular health, it would be helpful to define standards to identify these cells. With so many uncertainties, what is the “take home message” of the present study by Güven et al. (23)? In our view, this study highlights the potential shortcomings of attempting trans-}

**REFERENCES**


