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EDITORIAL COMMENT

Understanding the Role of Endothelial Progenitor Cells in Cardiovascular Disease, Coronary Artery Lesion Progression, and In-Stent Restenosis*

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Bone marrow-derived, circulating endothelial progenitor cells (EPCs) were first described by Asahara et al. (1) in 1997. They discovered that EPCs have regenerative capacities and play an important role in vessel wall homeostasis. Whereas animal studies have shown that these progenitor cells beneficially influence the repair of endothelial cells after injury and the progression of atherosclerosis (2,3), the role of EPCs in humans is less well understood.

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In subjects with cardiovascular risk factors, such as hypertension and diabetes mellitus, studies have shown that the number of circulating EPCs is reduced, and their function adversely affected (4,5). In contrast, elevated EPC levels were seen in patients that suffered an acute myocardial infarction (6) and patients that underwent a percutaneous coronary intervention (PCI) (7). Unfortunately, studies reporting on the number of circulating EPCs in patients with coronary artery disease (CAD) fail to show agreement. Some studies report that the EPC number is reduced in patients with atherosclerotic disease (8,9), whereas other studies report that EPC levels are indeed increased in CAD patients (10,11). There is accumulating evidence that a reduced number of EPCs is associated with the occurrence of ischemic cardiovascular events in patients with angiographically documented CAD (12,13).

Further assessment of circulating EPCs as surrogate biological markers might be helpful to identify novel therapeutic approaches to enhance endogenous vascular repair and favorably modify the progression of cardiovascular disease. The establishment of a healthy, functional endothelial layer may not only improve vascular homeostasis, but by abluminal secretion of anti-inflammatory and antiproliferative factors may also reduce neointimal formation following stent placement. Currently, the novel bioengineered Genous Endothelial Progenitor Cell Capturing Stent (OrbusNeich Medical Technologies Inc., Fort Lauderdale, Florida) coated with antihuman CD34+ antibodies attracting circulating EPCs is available in many countries for the treatment of patients with clinically significant CAD (14). Animal studies have shown that after only 60 min of incubation, a confluent monolayer of adherent CD34+ cells was formed covering the stent struts (15–17). In 2 small nonrandomized studies (HEALING–FIM [Healthy Endothelial Accelerated Lining Inhibits Neointimal Growth–First In Man] and HEALING II studies), the safety and efficacy of the EPC-capturing stent was demonstrated in patients with noncomplex coronary artery lesions. The multicenter, randomized TRIAS (TRI-Stent Adjudication Study) program is ongoing, in which the EPC capturing stent is compared to drug-eluting stents and bare-metal stents and in which EPCs before PCI will also be measured (18).

The number of circulating EPCs can be measured using fluorescence-activated cell-sorting analysis and standard gating techniques to detect surface marker expression. The EPCs' function can, in part, be measured by assessing their colony forming capacity in vitro (19). Modalities to increase the number or improve the function of EPCs may be promising in the treatment of atherosclerotic disease. Among these are physical exercise, administration of erythropoietin and treatment with statins (which enhance both the number and functionality of EPCs). In addition, local administration and systemic transfusion of vascular progenitor cells improves endothelial function and reduces atherosclerosis in animal models (20,21).

However, much remains to be clarified, including how these cells are characterized. For example, in 1 study (22) following angioplasty, level of circulating EPCs with a functional phenotype increases, whereas no increase is seen in the putative progenitor cells (CD34+KDR+) as characterized by surface marker expression, questioning whether these represent the same cell populations. In addition, in a recent large population study (23), hypertension, glycosylated hemoglobin, and plasma triglycerides were positively correlated with circulating EPC numbers, and the investigators speculated this may represent a protective, compensatory response.

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In this issue of *JACC: Cardiovascular Interventions,* Pelliccia et al. (24) examined the relationship between endothelial progenitor cells (CD34+KDR+ and CD133+KDR+), cells from the monocyte/macrophage lineage (CD45+CD14+), and angiographic outcome at 8 months. A total of 155 consecutive patients with stable angina underwent PCI with a bare-metal stent, and 20 healthy controls without CAD were also studied. At 8-month follow-up, the patients were subdivided in 3 groups based on their angiographic characteristics: patients without progression of CAD and without in-stent restenosis (n = 103), patients with progression of coronary atherosclerosis (n = 22), and patients with in-stent restenosis (n = 30). The number of cells in each cell population was prospectively measured the day before PCI and correlated with quantitative coronary angiographic assessments of in-stent restenosis and lesion progression on follow-up angiograms. No significant differences among the groups were found regarding the baseline clinical and angiographic characteristics although the overall and subdivided subject numbers were all small. Absolute numbers of EPCs, both CD34+/KDR+CD45– and CD133+/KDR+/CD45–, were higher in patients with in-stent restenosis than in patients without in-stent restenosis and controls. In addition, the number of CD14+/CD45+ cells was higher in patients with restenosis than in patients with lesion progression, patients in the stable CAD group, and in the control group. In contrast to previous (cross-sectional) studies, there was no significant difference in levels of EPCs between those with CAD and normal controls. Pelliccia et al. (24) concluded that patients who develop restenosis after bare-metal stent placement have higher baseline numbers of subpopulations of EPCs that incorporate into endothelial cells or play a role in arteriogenesis compared with controls and patients with either progression of coronary atherosclerosis or stable disease. Specifically regarding the development of in-stent restenosis, the investigators speculate that an abnormal engraftment of CD34+ and CD133+ EPCs causing excessive intima proliferation and in-stent restenosis may occur particularly among patients who have greater levels of EPCs at time of PCI. The results of Pelliccia et al. (24) are in contrast to those from previous reports (25-27) on patients treated with the EPC-attracting Genous stent. In 1 study, Duckers et al. (27) observed that decreased in-stent late lumen loss was associated with higher levels of circulating EPCs. These interstudy differences may be explained by distinctions in study designs or cell populations measured. In the study by Duckers et al. (27), EPCs were assessed 6 months following PCI, no bare-metal stents were used, and the cells identified as EPCs were 7AAD–/CD45+/CD34+/KDR+, so-called viable EPCs.

Considering these and other varied observations, one could conclude that despite meaningful investigations, the biology and clinical significance of EPCs in cardiovascular disease remain poorly understood. It is possible, for example, that the CD34+ population may be composed of precursors of both endothelial and fibroblast phenotypes. It is also illustrative that there is not a uniform unit of measure when assessing the number of circulating EPCs. In different studies, the number of EPCs has been expressed as number of cells per 1,000 white blood cells, percentage per 100 peripheral mononuclear cells, fluorescence-activated cell-sorting events per 10,000 counts, number of cells per 1 μl, or viable EPCs per 100 μl. Again comparing the studies by Pelliccia et al. (24) and Duckers et al. (27), even though both included similar patients with stable angina, there is a several-hundred-fold difference in the number of EPCs reported. It is also not know if it is the CD34+/KDR+/CD45– cells, the CD133+/KDR+/CD45– cells, or other cells that are responsible for colony forming in the functional colony forming unit (Hill) assay (4).

In summary, Pelliccia et al. (24) conclude that patients with restenosis have higher numbers of subpopulations of EPCs than control patients and patients with either progression of coronary atherosclerosis or stable disease. These results are appreciated and hopefully will be followed by observations from other investigators. For the future, several areas must be further pursued, including the characterization of bone marrow-derived circulating EPCs and subpopulations; the determination of factors that influence their number, function, and biological significance, both in healthy subjects and in patients with cardiovascular disease; and the standardization of measurements and units of measure to interpret results from different laboratories.

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Key Words: coronary artery disease ■ endothelial progenitor cells ■ percutaneous coronary intervention.