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EDITORIAL COMMENT

Endothelial Progenitor Obsolescence and Atherosclerotic Inflammation*

Chunming Dong, MD,
Lawrence E. Crawford, MD,
Pascal J. Goldschmidt-Clermont, MD, FACC
Durham, North Carolina

As early as in 1867, Cohnheim (1) noted that "all cells come from the bloodstream and therefore, in light of subsequent observations, from the bone marrow." The identification of endothelial progenitor cells (EPCs), extracted from human peripheral blood in 1997 by Asahara et al. (2), and subsequent studies have led to a significant paradigm shift in the field of vascular biology. The concept of arterial wall repair mediated by bone marrow-derived EPCs was born, providing an alternative to the local "response to injury hypothesis" for the development of atherosclerotic inflammation (Fig. 1) (3,4). According to this new theory, the arterial wall can deal fairly well with multiple circulating and local noxious stimuli, as long as the bone marrow-derived repair capacity, which includes competent EPCs and probably progenitors of other lineages, remains intact. Senescence of selected

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bone marrow pathways, a process that is accelerated by the passage of time in the presence of risk factors, can lead to the inability of the marrow to produce repair-competent EPCs and other marrow-derived progenitors (5,6). Hence, a reduced circulating EPC level has been proposed as a significant risk factor for cardiovascular disease. When the repair process becomes limiting, arterial cells, in particular endothelial cells (ECs), become senescent as manifested, for example, by the reduced length of chromosome telomeres (7,8). Thus, exogenous cells from young, but not old, bone marrows have the ability to engraft and rejuvenate arterial intimal cells in mouse models of atherosclerosis, as evidenced by the elongation of telomeres of these cells (6). The lack of ability for aging organisms to renew ECs exposed to noxious stimuli leads to endothelial dysfunction and consequent atherosclerosis genesis.

Dozens of publications have suggested a link among reduced circulating EPC levels, atherosclerosis, and its thromboembolic complications, such as angina, myocardial infarction, and percutaneous coronary intervention. Reduced levels of EPC also have been linked to disorders that produce a heightened cardiovascular risk, such as diabetes mellitus, renal failure, rheumatoid arthritis, and osteoarthritis, to name only a few (6–12). In this context, two important papers in the current issue of the *Journal* investigate the potential implications of circulating EPC levels in patients with type II diabetes mellitus complicated with peripheral vascular disease (PVD) (9) and the impact of aging on circulating EPC changes in young and old healthy individuals without major cardiovascular risk factors (10). These studies add a new dimension to our understanding of the putative role of circulating EPCs in the pathogenesis of vascular diseases, in particular in the context of diabetes and normal aging.

EPCs, ENDOTHELIAL REGENERATION, AND ATHEROSCLEROTIC INFLAMMATION

Several lines of evidence indicate that bone marrow-derived EPCs can be recruited to the ischemic limbs, brain, and to myocardial infarcts of mice, accelerating the re-endothelialization process (reviewed by Rafii and Lyden [11]). Endothelialization in adult dog thoracic aorta Dacron grafts was found to arise exclusively from transplanted bone marrow (12). The $CD34^+/VEGFR^+$ endothelial and hematopoietic precursors were recruited to the surface of left ventricular-assist devices in humans, rendering the surface nonthrombogenic (13). Furthermore, circulating EPCs could home to denuded parts of the artery after balloon injury (14). Rauscher et al. (6) demonstrated that bone marrow-derived EPCs engrafted the arterial wall of atherosclerotic apoE-deficient mice. These data demonstrate the plasticity of adult stem cells in differentiating into vascular cells and their capacity in the prevention and treatment of cardiovascular disease (15). However, aging in the presence of risk factors can lead to the progressive depletion of marrow cells that give rise to progenitors needed for arterial repair.

Because the repair process originating from the marrow is recruited by cytokines, growth factors, and other proinflammatory elements (such as monocyte inflammatory peptide-1-alpha, monocyte chemotactic protein-1, interleukin 1-beta, interleukin-6, tumor necrosis factor-alpha, C-reactive protein, and interferon-gamma), the delinquent repair process can lead to progressive amplification of the inflammatory signals due to the lack of a negative feedback loop. These signals, in turn, contribute to the aggravated inflammatory reaction (Th2-predominant) (4,16), further damaging the arterial wall (positive feedback loop) (Fig. 1). Hence, the elevation of markers not only reflects the

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From the Division of Cardiology, Department of Medicine, Duke University Medical Center, Durham, North Carolina. This work was supported by grants from the National Institutes of Health (P01 HL73042-02, 5R01 HL71536-08, and 1RO1 AG 023073-01) and the Doris Duke Charitable Foundation (20030028) to Dr. Goldschmidt-Clermont.

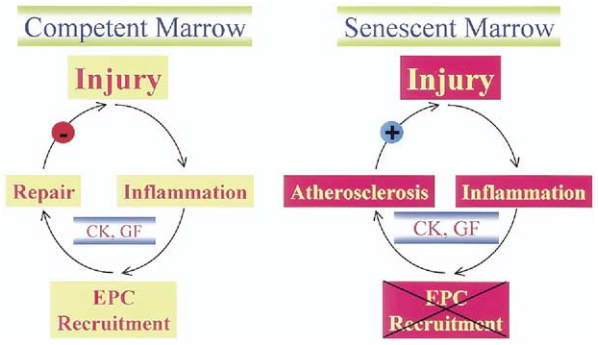

Figure 1. Effect of bone marrow obsolescence on arterial repair and inflammation. In the presence of a competent bone marrow capable of producing the type of progenitor cells for arterial repair (young marrow), inflammatory cytokines, growth factors, and other agonists contribute to the recruitment of repair cells. Once the arterial lesion is repaired successfully, the inflammatory markers subside (negative feedback loop). In contrast, if the bone marrow is unable to produce these progenitor cells, either because the pathways that are required for their production are deficient or because the produced cells are somehow incompetent, the inflammatory factors continue to increase, essentially indicating a persistent arterial lesion. The increase in inflammatory factors, in turn, can further promote arterial damage, with worsening of atherosclerosis and destabilization of atherosclerotic plaques (positive feedback loop). CK = cytokines and other inflammation agonists; EPC = endothelial progenitor cell; GF = growth factors. Adapted from Goldschmidt-Clermont et al. (4).

intensity of atherosclerotic inflammation but also may identify those individuals whose arterial repair is insufficient.

IDENTIFICATION AND CHARACTERIZATION OF EPCs AND THEIR PRECURSORS

Considering the growing evidence in support of circulating EPCs as a marker of vascular health, it would be helpful to define standards for measurements of this important variable. However, the molecular and phenotypic determinants of EPCs and their precursors remain largely unknown. Attempts to accurately characterize these cells have been hampered by several hurdles (17). Indeed, a considerable overlap exists among proteins expressed on the surface of angioblast-like EPCs, mature ECs sloughed from the vessel wall, and hematopoietic cells. Specifically, both putative EPCs with angioblastic potential and vessel wall-derived mature ECs may express similar endothelial-specific markers, including vascular endothelial growth factor (VEGF) receptor-2 (VEGFR2, kinase insert domain receptor [KDR], flk-1), Tie-1, Tie-2, vascular endothelial-cadherin, and CD34 (18–20). Hematopoietic stem cells express markers that also are found on EPCs and mature ECs, such as CD34, platelet endothelial cell adhesion molecule (CD31), Tie-1, Tie-2, von Willebrand factor, and VEGFR2 (21). Consequently, an array of different methodologies has been used to determine the number of circulating EPCs, including $CD34^+$, $VEGFR2^+$, $CD133^+$, $CD34^+/VEGFR2^+$, $CD34^+/CD133^+/VEGFR2$, $CD34^+/CD117^+/VEGFR2^+$ (mature EPCs), $CD34^+/CD31^-$, $CD34^+/P1H12^+$, $CD133^+/VE\text{-}cadherin^+$, $CD34^-/CD14^+$, $CD34^+/CD14^-$, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine-labeled acetylated low-density lipoprotein uptake/lectin binding, $cKit^+/Sca\text{-}1^+$, $cKit^+$, $CD49d^+$, $CXCR4^+$, and early- and late-outgrowth EPC colonies (22–29).

As a result, these variations in method have created confusion regarding data interpretation and may explain the inconsistent results obtained in different studies. Indeed, the conclusion by Heiss et al. (10) that normal aging resulted in functional deficits rather than depletion of EPCs is based on the lack of numerical changes of CD34/KDR (VEGFR2) or CD133/KDR double-positive cells and the decreased number of outgrowing ECs from the mononuclear fraction of the peripheral blood. Although the finding that the functional capacity of circulating EPCs declines with aging is intriguing, this conclusion may be challenged by the possibility that a subset of the cells that they surveyed, or even another group of bone marrow-derived progenitors capable of differentiating to ECs, may have been depleted, unbeknown to the investigators. The study by Fadini et al. (9), which quantified $CD34^+$ cells, defined as circulating progenitor cells, and $CD34^+/KDR^+$ cells, defined as EPCs, shows that PVD is associated with an extremely low number of EPCs, underscoring the notion that the complicating vascular injury in the presence of diabetes results in further decline in the EPC reservoir in the bone marrow and/or further impairment in EPC mobilization compared with diabetes alone. However, it remains to be determined whether changes in $CD34^+/KDR^+$ cells do fully reflect changes in EPCs capable of arterial repair and angiogenic activity.

RELEVANCE TO THE MANAGEMENT OF PATIENTS WITH ARTERIAL DISORDERS

Progress in managing atherosclerosis and related conditions, such as coronary artery disease, cerebral vascular disease, and PVD, has occurred through successive steps that include: 1) anatomical definition of the lesions (which has resulted in multiple techniques to image arterial lesions); 2) recognition of risk factors for the pathogenesis and their control (which, thus far, has the greatest impact on the disease process and its thromboembolic complications); 3) mechanistic insight with the characterization of the inflammation process (new markers for risk prediction have resulted from this advance); and 4) understanding of the arterial repair process that is needed to maintain arterial homeostasis and involves not only local reactions but importantly the recruitment of bone marrow precursor cells. Inadequate repair is particularly important because it may determine atherosclerosis initiation, progression, and lesion destabilization. Hence, the progressive obsolescence of the bone marrow may account for the powerful risk associated with aging (15).

The study by Heiss et al. (10) stresses the importance of aging relative to the dysfunction of EPCs and, hence, cells recruited from the marrow in situations of acute coronary syndromes, even in individuals without significant coronary risk factors, may be incapable of arterial repair, leading to

extensive destabilization of coronary vessels. Estrogens are known for their ability to stimulate the production of EPCs (30), but for aging women whose ability to produce these cells may be already challenged, the addition of hormone replacement therapy might lead to acute disruption of residual repair capacity. The data of Fadini et al. (9) suggest that diabetes mellitus impacts negatively on the ability of the bone marrow to mount the successful production of EPCs. Hence, the effect of diabetes on arterial disorders such as PVD may involve not only local effects on the arterial wall but also a deleterious consequence for pathways in the marrow that are responsible for producing an armamentarium of EPCs required for repair at various sites of the arterial tree. Statins—drugs known for their remarkable impact on the prevention of arterial thrombotic events—may protect the marrow from the impact of aging, even beyond what could be expected from the reduction of the cholesterol risk and consequent consumption of the arterial repair potential of individual patients (29).

It is still unclear at the present time whether new techniques will be successful in providing progenitor cells capable of arterial repair to patients whose intrinsic capacity has been eroded with aging and by risk factors. Such techniques may involve: 1) the provision of allogeneic progenitors that would reconstitute the repair potential of the affected patient; 2) the restitution of an intrinsic repair function through drug therapy and/or precursor cell processing; or 3) a combination of both approaches. Regardless of the outcome of the eventual breakthrough technology, this newest wave of EPC repair research is highly likely to have a substantial clinical impact on the cardiovascular epidemic worldwide.

Reprint requests and correspondence: Dr. Pascal J. Goldschmidt-Clermont, Division of Cardiology, Department of Medicine, Duke University Medical Center, Box 3845, Durham, North Carolina 27710. E-mail: golds017@mc.duke.edu.

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