Editorial

The Ponzo Effect

Endothelial Progenitor Cells Appear on the Horizon

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Can cell-based therapies be used to treat cardiovascular diseases that result from injury, inflammation, and the wear and tear of aging and cellular senescence? This is the premise of regenerative medicine. This emerging field has developed from advances in our understanding of cellular and molecular mechanisms contributing to cardiovascular disease in the post—“response to injury” era and, more directly, from the recent explosion of interest in stem cell biology and cellular plasticity. The field of stem cell biology has made enormous advances in recent years, with one example being the identification of adult multipotent progenitor cells capable of contributing to a variety of tissues (including endothelium and muscle). The recent demonstration that bone marrow–derived precursor cells can differentiate to cardiac myocytes and can improve cardiac function after myocardial infarction in animal models contradicts several decades of dogma about the nature of heart muscle lineage commitment and raises the (as yet unfulfilled) promise of regenerative therapies for myocardial infarction and heart failure. In parallel, regenerative medicine has set its sights on vessel wall events—in particular, atherosclerosis and angiogenesis—as additional targets for cell-based therapies. In this issue of Circulation, Szmitko et al concisely describe what is known about endothelial progenitor cells (EPCs) as potential tools in the setting of vascular disease. At the same time, it is worthwhile to consider what is not known about vascular progenitor cells and where the field must head if regenerative medicine is to achieve clinical applicability.

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What Are EPCs?

To address this question, it is first necessary to understand the general definition of progenitor cells and their antecedents, stem cells. In simple terms, stem cells are small populations of cells in adults that retain the ability to regenerate multiple cell types or lineages within a tissue or organ. However, the operational definition of a stem cell is much more difficult (and controversial); these cells must be able to proliferate in a self-renewing capacity and also to create multiple differentiated cell types under appropriate conditions. It is a challenge to demonstrate experimentally that a single putative stem cell possesses both of these properties. In addition, artifacts of in vivo manipulation confound interpretation of many studies presumed to demonstrate “stemness.” Suffice it to say, it is clear that stem cells exist during embryonic development, and it is probable that some populations of stem cells persist in adults, but their distribution and the diversity of lineages they may populate are yet to be determined.

Precursor (or “transit”) cells represent cells that are at varying points from stemness, with no (or at least less) potential for self-renewal. At the same time, precursor cells are programmed (or “fated”) toward particular lineages. Like stem cells, progenitor cells are operationally defined, and it is perilous to assign a progenitor designation without fully testing these attributes in a cell population. To make matters more complicated, it is necessary to prove that putative progenitors are not simply mature cells that have the capacity for transdifferentiation (that is, the ability to acquire some or all characteristics of another mature cell type). Thus, an EPC must not be able to perpetually renew and cannot itself represent a differentiated lineage but must, under appropriate cues, differentiate to a mature endothelial cell (and perhaps a limited number of related cell types). Since the landmark publication by Asahara et al describing the isolation of putative EPCs from humans, there has been a flurry of publications describing the isolation, functions, and physiological regulation of EPCs. What is it that these investigators are talking about?

First of all, it is likely that they are not all talking about the same thing. Cells carrying the designation “endothelial progenitor” have been isolated from the blood, bone marrow, skeletal muscle, and embryonic stem cells, using a variety of different surface markers (such as CD34, VEGFR2, and AC133) or simply by their adhesion and growth properties. Many of the cell populations identified in these studies have indeed been shown to acquire endothelial characteristics in vivo, such as incorporation into the luminal surface of blood vessels, uptake of acetylated LDL, and expression of endothelial cell surface markers. However, cells of this description are rare, and it is estimated that up to 12 L of autologous blood would be needed to obtain a therapeutically useful dose of human EPCs. In addition, recent evidence calls into question the origin and nature of at least some of these putative precursors. Rehman et al have isolated circulating cells in humans using techniques applied by many investigators in the field, and they show that these cells express definitive monocytic markers. Although these cells have some characteristics of
endothelial cells, they fail to express markers of definitive or stemlike endothelial cells, such as VE-cadherin, E-selectin, or CD34. At the same time, these cells are abundant sources of angiogenic factors, raising the interesting but unproven possibility that the therapeutic potential of putative EPCs may have more to do with the factors they secrete than with their ability to assume properties of the vascular endothelium. These studies do not in any way discount the elegant in vivo work of other investigators demonstrating incorporation of blood-derived cells into newly forming blood vessels and the acquisition of structural properties (such as the presence of Weibel-Palade bodies) that are typical of endothelial cells.6 The studies of Rehman et al7 do indicate that we need to know more about what discriminates circulating EPCs from other cells with similar behavior and more generally what steps stem cells take en route to becoming mature vascular endothelial cells, if that is indeed what they become.

**How Do EPCs Affect New Blood Vessel Formation?**

As indicated in the review by Szmitko et al,3 several groups have now shown that mobilization or transplantation of EPCs enhances new blood vessel growth in animal models. The general dogma holds that new vessel growth in the postnatal period occurs exclusively by the process of angiogenesis (that is, sprouting of new blood vessels from existing vessels), whereas during embryological development, vessels arise by both angiogenesis and vasculogenesis (formation of blood vessels and blood cells de novo from precursors in which no blood vessels previously existed). In the case of EPCs, investigators have shown incorporation of these circulating cells into newly forming vessels (for instance, in the border zone after myocardial infarction and in ischemic muscles9). This process has been termed, in my opinion incorrectly, “therapeutic vasculogenesis.”7 By all accounts, the morphology of vessels containing EPCs is typical of sprouting angiogenesis, and most key elements of vasculogenesis as classically defined are absent. In the broadest sense, the contribution of EPCs to new blood vessels in adulthood may represent a variant of typical angiogenesis (although there is no reason to discount the possibility that all angiogenic events in adults include EPCs as a component of vessel formation).

It is generally inferred that EPCs amplify vessel density by increasing the number of endothelial cells available to create new vessels. This assumption is predicated on data from aging or diabetic animal models and human studies in which angiogenic responses are attenuated,10,11 and the argument has been made that decreased endothelial cell migration and/or proliferation hinders angiogenic vessel growth. The observation that as many as 20% of endothelial cells in new blood vessels are likely of EPC origin12 supports the hypothesis that EPCs overcome a structural limitation in angiogenesis due to impaired migration and proliferation of mature endothelial cells. However, it is important to keep in mind that alternative or complementary functions of EPCs may account for their proangiogenic effects. EPCs may secrete paracrine factors that regulate the angiogenic response or may participate in specific cell-cell contact events that facilitate vessel sprouting and growth. Knowledge of how EPCs contribute to new vessel growth may have therapeutic value; for example, EPC-derived growth factors may be particularly valuable proangiogenic pharmacological tools.

**Do EPCs Have Clinical Utility?**

Although the review of Szmitko et al3 largely focuses on the therapeutic potential of EPCs, it is worth emphasizing that peripheral blood EPC counts may also have prognostic value for vascular disease. The number of circulating EPCs correlates inversely with major risk factors for coronary artery disease (in particular, smoking and family history)13 and overall Framingham risk score14 and is a better predictor of flow-mediated brachial reactivity than traditional risk factors for coronary disease.15 Although it is an unwieldy clinical marker at present, knowledge of EPC number or function (or an easily measurable index of these parameters) may eventually be useful for cardiovascular risk stratification. These studies also indicate that EPCs and other vascular progenitors may have regenerative functions that can be harnessed clinically in injury- and atherosclerosis-prone blood vessels. The recent enticing demonstration that vascular progenitors reduce atherosclerotic lesion formation in atherosclerosis-prone apoE−/− mice15 provides proof of principle for such a model, above and beyond any effects of EPCs on angiogenesis.

So what about EPCs and angiogenesis? Therapeutic angiogenesis for ischemic vascular diseases, using angiogenic growth factors, is an idea that is a decade old now. The randomized clinical trials testing this approach (reported in the past year) have included relatively small numbers of patients with modest and mixed, but still promising, clinical benefits. Most animal studies of EPCs have pursued the same general hypothesis, namely that EPCs will enhance angiogenesis in areas of ischemia or infarction to improve blood flow and clinical outcomes. Thus, EPCs have been tested (and shown to be beneficial) in animal models of myocardial infarction, stroke, and peripheral vascular disease.9,16,17 As with therapeutic angiogenesis approaches using growth factors (which have had a controversial and rocky course), there will be the concern of untoward consequences for EPC-based therapies—increasing the risk of retinopathy, tumor growth, myocardial infarction, and other consequences of pathological angiogenesis—until these are proven not to be adverse reactions toward these therapies. (Indeed, EPCs contribute to tumor angiogenesis in animal models.9) There will be other concerns as well; progenitor-based therapies raise issues of adverse effects such as infection, oncogenic transformation, and rheological consequences that are not anticipated by traditional pharmacological approaches.

Time will tell whether the introduction of human trials of EPC-based therapies represents an appreciation of the enormous potential for this new therapeutic modality or a failure to consider their dark side. In any event, their time is here. A small trial testing the effect of EPC-enriched bone marrow fractions for therapy of ischemic peripheral vascular disease18 is difficult to interpret—it is hard to know whether one should be encouraged by the modest but significant improvements in limb perfusion or concerned about the incidence of myocardial infarction (2 out of 25 patients). Following the history of
other therapeutic angiogenesis approaches, one can be sure that other studies, with varying degrees of invasiveness and rigor, will follow. What will they tell us about EPC-based therapies? The Ponzo effect refers to the difficulty of assessing the size of an object as it appears over the horizon. It is an apt metaphor for new therapeutic ideas such as the clinical application of EPCs. It is too soon to know whether EPCs will rise up to assume their place in the therapeutic armamentarium of cardiologists, neurologists, and other physicians who treat patients with vascular disease.

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References

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