Cell Therapy for Angiogenesis
Embracing Diversity

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“Alone we can do so little; together we can do so much.” —Helen Keller

The angiogenic effects of cell delivery were originally demonstrated by Asahara et al.2 using circulation-derived cells that were capable of assuming features of endothelial cells after brief periods of in vitro culture. These cells were referred to as endothelial progenitor cells (EPCs). This approach has been tested by Assmus and colleagues in the Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) study with intracoronary delivery of EPCs into the infarct-related coronary artery of patients after acute myocardial infarction.4 Concurrent with the clinical translation of this approach, the origin, biology, proliferative capacity, and therapeutic potential of EPCs have been questioned.

Consistent with the original cells described by Asahara et al, Rehman et al demonstrated that EPCs are of monocyte/macrophage origin and are capable of secreting angiogenic peptides.5 These peptides include vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8). EPCs were also shown to have a limited proliferative potential, a fact not consistent with in vitro generation of highly proliferative outgrowth endothelial cells (OECs), as demonstrated by Hebbel and colleagues.7 OECs were originally described as monomorphic, homogenous cells with distinct endothelial phenotype resulting from in vitro culture of circulating mononuclear cells. We demonstrated that these cells have distinct origins because the precursor of OECs comes from a CD14+ population of cells, whereas early EPCs are predominantly from CD14− precursors.8 Ingram and associates have extended these observations, suggesting that a hierarchy of true endothelial precursors exists in peripheral blood, umbilical cord blood, and perhaps vascular tissue.9,10 It remains to be seen whether the ultimate endothelial precursor is similar or identical to the multipotent adult progenitor cells (MAPC) as defined by Reyes et al.11

The present article by Yoon and Hur1 extends their previous studies that demonstrated angiogenic capacities of early EPCs and late EPCs or OECs. They test the hypothesis that delivery of a mixed population of early EPCs and OECs would have synergistic angiogenic effects. In vitro studies supported previous findings demonstrating the distinct origins and phenotypic differences between EPCs and OECs. These studies confirm that EPCs are heterogeneous and have CD14− and CD14+ origins, whereas OECs have a CD14− origin and that these CD14+ cells contain virtually all of the CD133+ and CD34+/KDR+ cells presumed to be markers of true endothelial precursors.5,12 In addition, the expression of cytokines from these cells differed as CD14+-derived cells produced more IL-8, VEGF, and matrix metalloproteinase-9 than did CD14−-derived cells that expressed more matrix metalloproteinase-2. Thus, these in vitro studies confirm the emerging paradigm that early EPCs are monocyte derived (and not truly endothelial) and of limited proliferative potential (and not progenitors), but are capable of assuming endothelial features (uptake of acetylated low-density lipoprotein and binding of Bandeiraea simplicifolia-lectin) and producing and secreting potent cytokines and growth factors. Thus, the term EPC is significant only in an historical rather than literal sense. The true endothelial precursor cell population (capable of generating OECs in vitro) is rare within the
circulation and likely originates from a subset of CD14⁻/CD34⁺/KDR⁺ cells that is not fully defined.

The in vivo studies by Yoon and Hur used delivery of labeled human cells to define the in vivo fate of CD14⁻ and CD14⁺-derived cells and early EPCs in immunodeficient mice. These studies suggest that there was differential distribution of cells for 7 days after delivery. CD14⁻-derived cells were more likely than CD14⁺-derived cells to be within a capillary network. After delivery of early EPCs, rare cells were seen in similar positions. Limitations to these studies include those inherent with the use of membrane dyes (including lack of stability and uptake by nondelivered cells by endocytosis, transfer, or fusion), the limited phenotypic (expression of VE-cadherin and KDR) and structural (position in muscle interstitium) analyses used to define the endothelial nature of delivered cells, as well as a lack of direct assessment of in vivo proliferation. Nevertheless, in vivo heterogeneity was demonstrated among the populations studied.

Because early EPCs have been shown to secrete potent angiogenic proteins and cytokines and OECs have been shown to be proliferative and reside more commonly in capillary networks, it was hypothesized that factors secreted from early EPCs may enhance the angiogenic capacity of OECs. In vitro analysis demonstrated that coculture of early EPCs or application of conditioned media from early EPCs stimulated the angiogenic phenotype of OECs including proliferation and tube formation in an IL-8- and VEGF-dependent manner. Delivery of mixed populations of early EPCs and OECs increased limb perfusion and limb salvage in a murine model of limb ischemia compared with either EPCs or application of conditioned media from early EPCs may enhance the angiogenic capacity of OECs. In vitro analysis demonstrated that coculture of early EPCs or application of conditioned media from early EPCs stimulated the angiogenic phenotype of OECs including proliferation and tube formation in an IL-8- and VEGF-dependent manner. Delivery of mixed populations of early EPCs and OECs increased limb perfusion and limb salvage in a murine model of limb ischemia compared with either population alone. This effect was greatest 14 to 21 days after delivery, suggesting a temporal dependence, and was associated with a greater detection of delivered cells detected, suggesting enhanced viability or residence of mixed populations.

This study adds to the growing understanding of cellular cooperation in biological approaches to angiogenesis. Angiogenic approaches including delivery of proteins or transgenes require the cooperative interaction of adult host tissue and circulating cells as well as progenitor cells to engender an angiogenic result. These cells include circulating monocytes, local endothelial and smooth muscle cells, and likely, circulation-derived precursors. Each of these cells may be required for a therapeutic effect. Delivery of EPCs likely has the advantage of delivering a potent source of regulated paracrine factors. It is unlikely that the only cells affected by these factors are the delivered cells. Most likely, resident endothelial cells and circulating progenitors are affected by these paracrine factors. As demonstrated in the article by Yoon and Hur, delivery of EPCs mixed with cells with a well-defined endothelial phenotype enhanced their angiogenic effects.

Although it may be tempting to conclude that the EPCs were the "builder cells" that directed the assembly of the OECs as "building blocks," data from other models suggest that this paradigm may be overly simplistic. In a rabbit vascular injury model, Griese and colleagues delivered genetically labeled OECs after balloon injury to a carotid artery. No genetic marker was detectable at 4 weeks, implying that none of the delivered OECs remained. Despite this, there was an impressive increase in overall arterial reendothelialization, suggesting that delivered OECs may have exerted their effects in a paracrine manner. In this model, we demonstrated a similar disconnect between the profound effect of delivered cells on endothelial-dependent vasoreactivity and neointimal formation in spite of a lack of long-term residence. Moreover, in vitro coculture studies suggest that IL-8 released by OECs promotes proliferation of mature endothelium. Thus, OECs can be added to the growing list of cells that may exert their beneficial effects at least in part in a paracrine manner on a number of cell types.

Why then might the EPC-OEC combination approach demonstrated by Yoon and Hur be more effective than delivery of each population alone? Perhaps it relates to the complex pattern of cytokines released by multiple (rather than single) cell types. Physiological angiogenesis involves many cellular components and multiple cytokines. Moreover, synergistic relationships between VEGF, fibroblast growth factor, and porcine insulin-like growth factor, among other cytokines, are well documented. Thus, this combination may provide a more diverse and coordinated pattern of cytokines and growth factors while providing cells that respond (at least temporarily) to these factors.

Several questions remain to be answered: What is the optimal cell combination for angiogenesis? Are "endothelialized" monocytes (EPCs) more effective than macrophages or monocytes? If so, which factors of culture modification might be important? What is the role of cell viability and proliferative capacity of delivered cells in this process? Alternatively, does the death of delivered cells affect angiogenesis? (Dead or dying cells may affect a healing or inflammatory process resulting in angiogenesis.) With the realization that multicellular cooperation in addition to coordinated protein expression is required for effective angiogenesis, what will be its impact on translational approaches? Would a mixed cell-delivery approach be feasible for clinical trials? OECs require 2 to 3 weeks in culture for differentiation and amplification. Thus, if cells are not prepared beforehand, this approach would require advanced planning but may be applicable for chronic ischemic syndromes. Unless autologous cells were cultured and stored, acute approaches such as that in TOPCARE-AMI would not be feasible. Alternatively, this approach may be enhanced by the use of freshly isolated cells or of cells after acute genetic or culture modification.

Rather than a call for rapid clinical translation, this article by Yoon and Hur is a call for more coordinated efforts to understand the mechanisms responsible for the therapeutic effects of cell delivery. Preclinical studies such as this continue to provide insights into the diversity and complexity of the angiogenic process. These studies may lead to more straightforward translational approaches. An example of this process is the realization that Akt-transduced mesenchymal stem cells affect cardiac repair by the release of paracrine factors. Isolation of these factors may lead to alternatives to cell delivery. Ultimately, these complex biological strategies should be tested in patients by diverse and cooperative teams.
of clinicians and scientists heralding a new phase of biological therapeutics for cardiovascular disease.

Disclosure
Dr Simari is a site investigator and nonpaid steering committee member on a multicenter clinical gene transfer trial sponsored by Corautus Genetics, Inc.

References

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