Showing up Isn’t Enough for Vascularization
Persistence Is Essential

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When Woody Allen claims that “80% of success is showing up” he underestimates the importance of sticking around. Case in point: formation of functional vessels in the wake of an ischemic injury by adoptively transferred cells. Although paracrine factor release, survival signaling, cell–cell communication, and endogenous cell recruitment all undoubtedly contribute to the process, an elegant study by Ziebart et al1 in this issue of Circulation Research demonstrates persistence of adoptively transferred cells is also a critical facet of successful revascularization.

Potent vascularization mediated by adoptively transferred endothelial progenitor cells (EPCs) has been widely documented and is now part of our collective regenerative therapy dogma.2–3 The initial isolation of EPCs as CD34+ hematopoietic progenitors more than a decade ago prompted intense research throughout the world to delineate the mechanistic basis for vessel formation by these cells. Complexities abound in the process of EPC-mediated vasculogenesis involving a multistep process of recruitment, homing, attachment and migration, proliferation, plexus formation, and, eventually, vessel stabilization. Along the way to revascularization, bidirectional communication between circulating EPCs recruited to ischemic injury sites and cells residing in the compromised target tissue. The multifaceted nature of this process and the multiple players involved obscure the relative contributions of recruited EPCs versus resident tissue populations to establish mature vasculature. This is a pivotal issue for myocardial regenerative therapy, because the ultimate efficacy of rebuilding healthy and functional contractile tissue depends on establishment of an integrated and stable vascular network.

Incorporation of bone marrow–derived EPCs into neovascularization has been demonstrated by transgenic approaches with mice expressing reporter constructs driven by endothelial lineage–specific promoters.5–7 However, the necessity for engraftment of donated EPCs in newly formed vascular network to maintain structure and function was unresolved. Local mature endothelial cells and attendant paracrine factors mediating survival and differentiation contribute significantly to the process, raising the question of whether these transferred EPCs become an integral part of the new vascular network or alternatively acting primarily as the trigger cells to prompt endogenous repair.

To test whether salutary effects of EPC adoptive transfer are tied to stable engraftment into vasculature, Ziebart et al assessed the consequences of cell therapy using a cleverly genetically engineered EPC population designed to commit inducible suicide. By subsequently killing off the donated EPCs several days after delivery, the requirement of engrafted cells for stable revascularization could finally be determined. This bit of suicidal genetic mischief came in the form of a lentivirally delivered thymidine kinase (TK) gene that acts on gancyclovir, turning the drug into a cytotoxic agent. Gancyclovir treatment was found effective, nontoxic to normal cells lacking the introduced TK gene, and did not mediate “bystander” effect of nearby normal cells being caught up in the suicidal demise of their genetically tweaked neighbors. With the self-annihilating EPCs in hand, Ziebart et al introduced them into 3 distinct venues for determination of their role in neovascularization: (1) myocardial infarction, (2) Matrigel plug angiogenesis, and (3) hindlimb ischemia. In all 3 cases, administration of gancyclovir impaired aspects of vascular structure and/or function in mice with engrafted TK-expressing EPCs but not the control green fluorescent protein–expressing EPCs. In the myocardial infarction model, a significant decline in myocardial function, as determined by ejection fraction, was also noted. The authors conclude that engraftment and persistence of the donated EPCs is necessary for preservation of neovascularization.

The puzzle piece of donated cell engraftment can now be added to the emerging mechanistic picture of vascular regeneration and repair. With such marked effects on vascular network stability and myocardial function resulting from genetically engineered death, how much of the newly formed vasculature is directly impacted by the induced suicide of the donated cell population? The results of the study do not provide a clear answer, because all we are told is that the donated cell population accounts for ≈1.5% of the cells in the border zone of the infarct region in control myocardial samples harvested at 4 weeks. However, the gancyclovir treatment was started at 2 weeks after infarction when the relative percentage of donated cells was possibly much higher. Thus, this experimental system provides a snapshot of donated cell depletion at a single point in time that could be expanded to understand the participation of donated cells in long term. How long after delivery of the transferred cells until gancyclovir treatment does not matter anymore? Would gancyclovir treatment at 4 weeks, when the donated population accounts for 1.5% of the total cells, lead to deterioration of vascular stability comparable to that documented at the
earlier time point? Capillary leakage was observed in the hindlimb ischemia model. Assuming similar effects occur in the myocardium after induced cell suicide what role did local tissue edema and inflammatory cell infiltration play in accelerated deterioration of myocardial function irrespective of capillary density loss? Vascular degeneration may only tell part of the story in the loss of hemodynamic performance and structural remodeling in the myocardial infarction model.

The provocative findings of this study raise additional questions that need to be answered. How much of early neovascularization is a cell numbers game? The postulate that persistence of donated cells promotes vascular maturation suggests that more donated cells may lead to either enhanced plexus formation and/or maturation. Would a higher number of donated cells lead to more profound effects on gancyclovir treatment, or is there a maximal load of cell engraftment beyond which additional cells become irrelevant? Transdifferentiation of donated EPCs was found to be a relatively rare event at <0.1% of the myocyte population, but the true nature of such plasticity and contribution to recovery could be unambiguously determined by engineering the TK gene to be driven by a cardiomyocyte-specific promoter, thereby enabling the selective elimination of donated cell–derived myocytes. The precision of such maneuvers depends on the lack of bystander effects, wherein suicidal cells with functional gap junctions may offer a “kiss of death” to their innocent neighbors. Although this sort of collateral damage was not a concern assessed by Ziebart et al under in vitro coculture, similar cell-independent specificity would need to be verified in vivo.

Studies in myocardial regeneration have highlighted a critical role for paracrine factors and survival signaling in regenerative processes. The recent surge in therapeutic use of adoptively donated EPCs to accelerate vasculogenesis in the wake of ischemic injury has prompted researchers to examine how the process can be enhanced. Connections between survival signaling and paracrine factors are inextricably intertwined, and genetic engineering to deliver EPCs (the “substrate”), together with paracrine factors (the “ligand”), augments the efficacy of vasculogenesis. Exploring persistence of donated cells in such enhanced regeneration systems using the suicide gene approach described by Ziebart et al could provide valuable understanding of whether these supplemental enhancements work by increasing donated cell numbers and persistence, enhancing recruitment of endogenous repair processes, or (as seems most likely by cooperation) a combination of the two. The authors appropriately caution that the participation of paracrine mechanisms is intimately tied to some of the effects they observe. Thus, circulating cells homing to the region of injury need to persist but also depend on a symbiotic relationship with local resident cells to mediate repair that involves elaboration of paracrine factors leading to potentiation of survival, proliferation, and differentiation.

Having demonstrated that persistence pays off for donated EPC vasculogenesis, we now have one more consideration that needs to be juggled in optimizing design of a therapeutic approach. We will eventually figure out how much persistence is required of donated EPCs to form mature functional vessels with the cooperative help of sufficient endogenous vasculogenic cells. To paraphrase a Japanese proverb, the findings of Ziebart et al show that “New vessels grow on the tree of persistence.”

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References


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