R
evascularization of the myocardium, or growing new blood vessels, has become an important therapeutic target to relieve tissue ischemia caused by vascular disease and restore cardiac function. Recent experimental and clinical investigations have established the feasibility of using recombinant proteins or gene transfer of angiogenic factors to facilitate neovascularization and augment collateral development in ischemic tissues.1 By traditional definition, the growth of new vessels occurs via angiogenesis or vasculogenesis. Angiogenesis is defined as the formation of new vessels by a process of sprouting from preexisting vessels. It is characterized by dedifferentiation of endothelial cells, dissolution of basement membranes, proliferation and migration of the cells, and reestablishment of new endothelium-lined capillary tubes. Vasculogenesis refers to the primary in situ differentiation of endothelial cells from mesodermal precursors and subsequent rearrangement into a primary capillary plexus.2 Vasculogenesis was originally thought to be restricted to embryonic development, but intriguing research over the last few years has pointed to alternative mechanisms for postnatal neovascularization.

Asahara and colleagues3,4 published studies reporting a process that they termed postnatal vasculogenesis, involving circulating endothelial progenitor cells (EPCs). They isolated putative EPCs from human blood using antibodies to two antigens that are shared by angioblasts and hematopoietic stem cells: CD34, which is expressed by hematopoietic stem cells but is lost during differentiation, and Flk-1, a receptor for vascular endothelial growth factor that is expressed by early hematopoietic stem cells and endothelial cells but ceases to be expressed during hematopoietic differentiation. When plated on fibronectin, the mononuclear EPCs from plasma formed tube-like structures, a morphological phenomenon characteristic of angiogenesis in vitro. In addition, these cells expressed the endothelial lineage markers CD34, CD31, Flk-1, Tie-2, and E-selectin. When the EPCs were labeled with fluorescent dye and injected systemically into mice or rabbits with experimentally induced hind-limb ischemia, the labeled cells were found incorporated into foci of neovascularization.3 Additional studies used bone marrow transplants from mice expressing lacZ under the control of Flk-1 or Tie-2 endothelial-specific promoters. X-gal-stained EPCs could be identified in blood vessels in various models of physiological and pathological neovascularization.4 There was some controversy associated with the initial reports on EPCs; however, these observations have since been verified by several laboratories.5–7

In another fascinating and controversial study, Maniotis et al8 coined the term vasculogenic mimicry to describe the formation of vascular conduits by tumor cells in uveal melanomas. In this study, morphological evidence was presented documenting the existence of periodic acid-Schiff–positive matrix channels surrounded by tumor cells with erythrocytes frequently present in the middle of the channels. Injection of fluorescent dyes showed that the channels formed a complex, branched network. In vitro, the highly invasive melanoma cells formed tube-like structures and contracted collagen gels, again reminiscent of models of endothelial cell angiogenesis. This study has generated a great deal of controversy, eliciting two commentaries and one response from authors in the American Journal of Pathology9–11 as well as a special session in a recent Keystone Symposium on angiogenesis. However, the presence of tumor cell channels has been noted in other studies,11 and, in fact, for many years an epithelial tumor cell line (ECV304) was mistaken for endothelial cells on the basis of the ability to form tubes and express factor VIII.12 These data blur the distinction between cell phenotypes. Even in normal physiology, nonendothelial cells can take on endothelial-like roles. For example, during placental development, fetal cytotrophoblast cells invade the maternal vasculature, masquerading as endothelial cells.13 At the very least, these studies suggest that we may need to revise our definitions of vasculogenesis to include these alternative means of developing vascular channels.

In this issue of Circulation Research, Moldovan et al14 report a unique paradigm for the compensatory vascularization of ischemic myocardium. They have created transgenic mice that overexpress the chemokine monocyte chemotactic protein-1 (MCP-1) under the control of the α-cardiac myosin heavy chain promoter to target MCP-1 expression to the adult heart. Cardiac overexpression of MCP-1 results in macrophage infiltration into the heart, and the mice suffer a thrombotic occlusive arteriolar vasculopathy that results in ischemia, interstitial fibrosis, ventricular dilation, and heart failure.15 The invading macrophages drill an extensive network of periodic acid-Schiff–positive tunnels within the myocardium. The tunnels and macrophages stain positive for mouse macrophage elastase, a protease with activity against a variety of matrix substrates. Most interestingly, many of the

---

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

From the Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada.

Correspondence to Michelle P. Bendeck, Department of Laboratory Medicine and Pathobiology, University of Toronto, Medical Sciences Building, 1 King’s College Circle, Room 6217B, Toronto, Ontario M5S 1A8 Canada. E-mail michelle.bendeck@utoronto.ca

(Circ Res. 2000;87:341-343.)

© 2000 American Heart Association, Inc.

Circulation Research is available at http://www.circresaha.org
tunnels seem to contain erythrocytes. The tunnels are not lined by endothelium and do not stain for endothelial markers such as endothelial NO synthase, platelet-endothelial cell adhesion molecule-1, and THY-1, so they are easily distinguished from mature capillaries.

The authors have postulated that the tunnels are colonized by circulating EPCs and present data to support this hypothesis. The tunnels occasionally contain cells that stain positive for Thy-1, a marker of hematopoietic stem cells. However, the strongest data were obtained by performing ectopic transplantation of the MCP-1 transgenic mouse donor hearts into recipient mice engineered to express β-galactosidase, presumably derived from circulating endothelial precursors that seeded and localized on the inner surface of the macrophage-drilled tunnels. This is a very exciting study that could lead to a paradigm shift in our thinking on the mechanisms of neovascularization in ischemic tissue. However, there are some caveats and several important questions that remain to be answered. One limitation of these studies is that the origin and nature of the cells lining the tunnels are not entirely clear. On the basis of the evidence presented in this study, we cannot rule out the possibility that the X-gal–labeled cells colonizing the tunnels are derived from sprouting capillaries that invade or connect with the tunnels, because all cells of the endothelial lineage synthesize β-galactosidase in the transgenic mouse recipients. More conclusive evidence would be provided by performing heart transplants into mice with bone marrow transplants for Tie-2 and β-galactosidase, because under these conditions, β-galactosidase expression should be restricted to EPCs originating in the marrow. Moreover, it is also possible that the cells lining the tunnels are resident macrophages that assume the morphological appearance of endothelium, and these cells might not assume full endothelial cell function.

To restore cardiac perfusion, the tunnels must eventually connect to the existing microcirculation and evolve into functioning blood vessels. There is no evidence in the present study that such connections evolve. Furthermore, a blood vessel is not just a tunnel in the interstitium but instead has important functional properties related to anticoagulation and regulation of tissue perfusion. The authors have presented evidence of colonization of the tunnels by immature EPCs; however, we do not know whether these cells will differentiate into an intact functional endothelium with normal permeability barrier, anticoagulant, fibrinolytic, and vaso-regulatory functions. In fact, even the previous studies of EPCs have not demonstrated that these cells differentiate sufficiently to fulfill all these functions. Another question is whether pericytes and smooth muscle cells will be recruited to surround the neovessels. This is an important step in vascular development and ensures that the vessels are able to respond to hemodynamic forces, including blood flow and blood pressure, to regulate perfusion.

Nevertheless, these data may lead to new possibilities for therapy. Indeed, recombinant protein and gene therapy for myocardial revascularization are presently under investigation. However, a potential hurdle to the success of these therapies is the status of the endothelium in the ischemic region. A compromised endothelium will not proliferate or migrate as well as healthy endothelium, and this may limit the degree and extent of neovascularization that can be achieved through stimulation with classical angiogenic factors. Thus, the delivery of activated macrophages to form tunnels in the myocardial interstitium may provide an attractive alternative or adjunct therapy, creating natural channels along which endothelial cells can migrate or seed from the blood. The formation of macrophage tunnels could be viewed as molecular transmyocardial revascularization.

At this point, we know very little of the mechanisms controlling macrophage tunneling in the myocardium. Studies in other systems have shown that MCP-1 alone does not affect mouse macrophage elastase production. The present study suggests that local hypoxia may be an important cofactor for MCP-1. In addition, other proteinases may be involved in the tunneling response. It will be interesting to discover whether other angiogenic factors present at high levels in ischemic tissues (vascular endothelial growth factor, fibroblast growth factor-2, and angiopoietin-1) potentiate the macrophage-tunneling response.

In conclusion, the data presented in this interesting study suggest another mechanism in addition to the secretion of angiogenic factors by which macrophages may participate in the elaboration of new blood vessels. Of course, we must also remember that the most important question remains to be addressed: does the establishment of macrophage-derived tunnels actually improve myocardial function? However, there is little doubt that this study will provide a channel for a substantial body of research into the fascinating phenomenon of endothelium-independent revascularization.

References

Bendeck

Macrophages Facilitate Revascularization

343


Key Words: myocardium • angiogenesis • vasculogenesis • macrophage • matrix metalloproteinases
Mining the Myocardium With Macrophage Drills: A Novel Mechanism for Revascularization
Michelle P. Bendeck

Circ Res. 2000;87:341-343
doi: 10.1161/01.RES.87.5.341

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/87/5/341