Hypoxia is a common feature of many diseases, including myocardial infarction, cerebral ischemia, pulmonary hypertension, and cancer. Thus, understanding the role of hypoxia in the pathogenesis of ischemic disease has significant therapeutic implications. Following ischemic injury, the growth of new blood vessels, neovascularization, is critical to maintain tissue reperfusion and homeostasis. Neovascularization occurs via 2 primary mechanisms: angiogenesis, the sprouting of new vessels from preexisting resident endothelium, and vasculogenesis, the organization of progenitor cells into vascular structures. Vasculogenesis was initially defined strictly as a developmental process. However, the characterization of bone marrow–derived progenitor cells (BMCs), which are able to differentiate into vascular cells, has suggested that vasculogenesis may also occur in adults. The remarkable ability of BMCs to contribute to vessel formation suggests a potentially beneficial role for these progenitor cells in regenerative medicine. Indeed, when BMCs are injected into animal models of ischemia, they “home” to sites of injury, migrate into tissues, and are associated with restoration of blood flow.

Mobilization of BMCs has been reported to have beneficial effects after myocardial infarction and arterial injury. Moreover, recent clinical trials reveal promising results using BMC injection as a treatment for myocardial infarction.

Previous studies have shown that BMCs are rapidly mobilized and recruited to sites of vessel injury. There is keen interest in determining which stimuli cause BMCs to home selectively to areas of ischemia. Recently, a molecular link between hypoxia and BMC mobilization has been reported involving the transcription factor hypoxia-inducible factor 1α and the chemokine stromal derived cell factor-1 (SDF-1). Hypoxia-inducible factor 1α, stabilized during hypoxia, up-regulates endothelial cell SDF-1 expression that, via its selective receptor CXC chemokine receptor-4, recruits BMCs to hypoxic areas. Because most animal models of ischemia involve inflammation in addition to hypoxia, it is unclear to what extent hypoxia alone is capable of recruiting BMCs to the vessels. Furthermore, an important area of controversy is whether BMCs transdifferentiate into endothelial cells or, instead, serve a paracrine function by secreting proangiogenic factors.

In this issue of Circulation Research, O’Neill et al address these questions by studying BMC recruitment and function in angiogenesis induced by hypoxia. In this study, they irradiated wild-type mice and transplanted them with bone marrow obtained from genetically engineered mice with BMCs expressing green fluorescent protein. Once the BMCs engrafted, mobilized cells could easily be identified in tissues by looking for the fluorescent label. Perhaps the most valuable contributions of this article are the animal model of systemic hypoxia and the high-resolution imaging of the spinotrapezius muscle. Their mouse model of systemic hypoxia excludes the effect of injury-induced inflammation, allowing for exclusive examination of hypoxia as a stimulus for BMC recruitment and angiogenesis. The use of whole-mount immunohistochemistry enables immediate visualization of all stained components within a thin layer of spinotrapezius muscle, thereby facilitating the identification, quantification, and localization of stained cells with more resolution than previous models. The major advantages of this model as compared with analysis of tissue sections include a much larger sample size in terms of BMC numbers, spatial information on the location of BMCs within the vascular bed, and more effective perfusion to remove BMCs that are nonadherent or weakly associated with the capillary luminal surface. Using this original and convenient approach, the group studied more than 10 000 capillaries and more than 8000 BMCs. They showed that BMC mobilization enhances hypoxia-induced angiogenesis, but most importantly they demonstrate that these BMCs do not incorporate and transdifferentiate into the newly formed capillary vessels.

Another novel finding in this study was the presence of BMCs in muscle tissue under normal physiologic conditions. The authors describe 2 distinct morphological populations of resident BMCs: round versus elongated cells (Figure). Round BMCs express the monocytic markers CD45 and CD11b, in contrast to elongated BMCs. In addition, elongated BMCs are 3 times more likely to be perivascular than rounded BMCs under basal conditions. These phenotypic differences most likely delineate distinct functional capabilities. At present, it is unclear whether these round cells are derived from the elongated cells. Depending on the severity of tissue damage, these resident BMCs may be sufficient for the local and immediate response to tissue injury and repair, bypassing a...
systemic BMC mobilization. An important future question is whether these resident BMCs are tissue specific or, instead, are universal tissue progenitors.

Another novel finding in this article is the reorganization of these resident BMCs in response to systemic hypoxia, without an overall increase in their tissue density. After 21 days of systemic hypoxia, the authors demonstrate a 13% increase in capillary density but no change in overall BMC density in the muscle tissue. This result suggests that hypoxia alone is not sufficient to initiate a systemic BMC mobilization. However, the density and localization of round BMCs significantly changed. Indeed, hypoxia induced a 25% increase in round BMC number and stimulated their accumulation in the perivascular area (Figure). Thus, hypoxia may, instead, play a role in the local reorganization of muscle-resident BMCs. It is known that BMC mobilization from the bone marrow into the bloodstream occurs in response to elevated serum levels of vascular endothelial growth factor, SDF-1, and various growth factors, such as granulocyte colony stimulating factor (G-CSF) and granulocyte–monocyte stimulating factor (GM-CSF). Here, O’Neill et al show that hypoxia alone is not enough to stimulate BMC mobilization and that systemic hypoxia appears to induce a local redistribution of tissue-resident BMCs.

During the last decade, some studies have suggested that BMCs are endothelial progenitor cells, capable of incorporating and differentiating into vessel-like structures, whereas other data suggest that they act solely in a supportive paracrine manner to release cytokines and to stimulate the growth of resident adult endothelial cells. Here, O’Neill et al provide evidence that BMC transdifferentiation does not occur in response to systemic hypoxia following GM-CSF–induced BMC mobilization. Transplanted mice treated with GM-CSF were compared in response to systemic hypoxia with mice untreated with GM-CSF. Although the number of round BMCs significantly increased (27%) with GM-CSF and was associated with a higher level of neovascularization (23%), no BMC incorporation was observed in the neovasculature. O’Neill et al conclude that systemic hypoxia does not stimulate GM-CSF–mobilized BMCs to transdifferentiate. In agreement with this report, Ziegelhoeffer et al, using a mouse ischemic hindlimb model, found that BMCs do not incorporate into growing collateral blood vessels but do localize perivascularly (Figure, local ischemia). This implies that circulating BMCs may represent a pool of cells secreting “supportive cytokines” during vascular growth processes. Recently, the investigation of paracrine capacities of marrow-
derived cells revealed that endothelial progenitor cells are indeed a potential source of cytokines such as vascular endothelial growth factor and GM-CSF.16

One way of reconciling the conflicting data in the literature is to consider the severity of vascular damage. In response to moderate tissue injury, BMCs may play a supportive role in angiogenic self-repair via secretion of proangiogenic cytokines, and transdifferentiation may not be required (Figure, local ischemia). In contrast, in the case of a severe vascular injury, degeneration and necrosis of the injured tissue may stimulate the transdifferentiation of BMCs during vasculogenesis. The nature and the severity of the injury appear to be important determinants of BMC behavior. The response of BMCs may also be governed by the intrinsic nature of the damaged tissue. For example, it is probable that mechanisms necessary to repair the brain or the heart are different from those required for the skeletal muscle response to hypoxia. Specifically, the release of cytokines may be useful to stimulate proliferation of cells with mitotic capabilities but have little effect on terminal cells such as neurons or cardiomyocytes.

Determining the functional properties of different BMC populations in individual tissues in response to particular stimuli is an important challenge that could possibly lead to the selection of appropriate BMCs for cardiovascular therapy. Based on their specific and selective recruitment to areas of injury, these cells could be ideal vehicles for specific drug delivery and the activation of desirable repair mechanisms.

Acknowledgments
This work was supported by NIH grants HL64839 and HL62826 (to B.C.B.). M.M. was supported by Medical Scientist Training Program grant T32 GM07356.

References

Key Words: stem cells • angiogenesis • hypoxia • bone marrow–derived cells
Tissue-Resident Bone Marrow–Derived Progenitor Cells: Key Players in Hypoxia-Induced Angiogenesis
Gwenaele Garin, Marlene Mathews and Bradford C. Berk

Circ Res. 2005;97:955-957
doi: 10.1161/01.RES.0000193566.65262.d8
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 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/97/10/955

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/