The field of progenitor cell therapy appears to be poised to transform the management of many cardiovascular diseases. In particular, the use of bone marrow-derived mononuclear cells (BM-MNCs) and endothelial progenitor cells (EPCs) have shown indications of improved cardiac function post–myocardial infarction (MI), with a recent metaanalysis showing a highly significant mean improvement in global ejection fraction of 3% compared to control. The overall positive results from these early trials raise 2 important questions. The first is whether cell therapy really works, in other words, whether the results of these smaller trials, which generally enrolled less than 100 patients, will be reproducible in larger pivotal studies. A study currently being planned by Zeiher and colleagues (A.M. Zeiher, personal communication) will help answer this question. The second question is whether we can do better, either by selecting a more active subset of highly regenerative progenitor cells, or by enhancing progenitor cell activity before delivery. Several clinical studies exploring these strategies are already planned or underway, including our own ENACT-AMI (eNOS and Cell Therapy) D Acute MI trial. To inform further clinical studies, it is also critical to better understand progenitor cell biology, including the mechanisms by which they exert their function in vivo, as well as the genomic and proteomic interactions that underlie their survival, homing, and differentiation.

In this issue of Circulation Research, Kränel et al2 demonstrate a novel pathway that may contribute importantly to EPC function and provide a potential marker of regenerative activity. They report that the kinin B2 receptor (B2R) was highly expressed by CD34+ and CD133+ MNCs, as well as in culture-selected “early growth” EPCs. Bradykinin (BK), a natural agonist for B2R, was shown to be a potent chemoattractant for the EPCs, and BK-responsive cells exhibited a more pronounced angiogenic phenotype. These effects of B2R activation involved signaling via the PI3K/Akt/eNOS pathway, because B2R blockade led to a similar reduction in migration as with inhibition of either PI3K or eNOS. Kränel et al also showed a reduction in the number of B2R-positive EPCs from patients with stable angina and acute MI, with an associated reduction in their migration toward BK. Finally, they showed that EPCs from B2R-deficient mice had a reduced ability to stimulate neovascularization in a mouse hindlimb ischemia model.

The finding that B2R expression and BK-responsiveness predicted progenitor cell function has potentially important experimental and clinical relevance. Kränel et al2 showed that cells selected by their ability to migrate to BK had higher expression of endothelial markers, greater in vitro angiogenesis and greater secretion of paracrine factors compared to those not responding to BK. However, they did not test whether BK-migrating cells indeed exhibited greater efficacy in the in vivo hindlimb ischemia model and, although they demonstrated that BM-MNCs from transgenic mice deficient in B2R had impaired neovascularization capacity, the authors were unable to show a sustained increase in perfusion past 1 week in response to the wild-type cells.

If differences in the regenerative capacity between the BK-responsive and -nonresponsive EPCs are confirmed in further preclinical studies, B2R might represent a useful selection marker for a more highly regenerative cell. With a recent study demonstrating the critical influence of cell processing procedures on the clinical benefit of cell therapy post-MI, investigators are acutely aware of the importance of verifying viability and functionality of administered cells in the design of clinical trials of autologous cell therapy. Currently, the relatively high prevalence of CD34/KDR- or CD133/KDR-positive EPCs in the administered BM-MNC population is being proposed as a potential explanation for the apparent clinical benefit. Similarly, selection of cells based on migration to BK and/or B2R expression might improve our ability to identify highly effective cell populations. However, this might be confounded by the presence of cardiac disease or risk factors, because EPCs from patients with acute MI or stable angina showed reduced expression of B2R compared to those of healthy controls in the present study. Thus, it remains to be confirmed to what degree these factors impact on BK-responsiveness or neovascularization capacity.

One of the caveats of autologous cell therapy is that the EPCs are derived from the very same patients with cardiovascular disease who need to be treated, and there is an inverse relationship between the number and function of the EPCs and the cardiac risks of the patients (based on age, cholesterol, diabetes, etc). Oxidative stress has been suggested to be at least partially responsible for progenitor cell dysfunction, likely through disruption of the PI3K/Akt/eNOS signaling pathway. Strategies aimed at enhancing this pathway have shown some promising results. Culturing cells in the presence of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) or a PPAR-γ agonist (pioglitazone), agents that activate Akt through different mechanisms, have been shown to improve EPC function. In

“B2 or not B2?”

Kinin Receptors and Endothelial Progenitor Cell Dysfunction

Michael R. Ward, Jessie Lavoie, Duncan J. Stewart

© 2008 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org
DOI: 10.1161/CIRCRESAHA.108.189084
addition, increasing the bioavailability of NO, either exogenously or by eNOS overexpression has led to improved migration, neovascularization, or endothelial repair. No doubt there are other unknown pathways that may be critical to the regenerative function of these cells and that may be uncovered only by using unbiased genomic or proteomic approaches, for example, by assessment of differential phosphoproteomic signatures in cells from patients with vascular disease. Nonetheless, a better understanding of the underlying mechanisms responsible for EPC dysfunction will provide the basis to develop better strategies to improve the efficacy of autologous cell therapy. The study of EPC function as a surrogate for cardiovascular risk has generated much interest, although the complexity of EPC definitions and isolation techniques have impeded the evaluation of this as a diagnostic test. Because progenitor cell B2R expression can easily be measured by flow cytometry, this could potentially be evaluated as a marker of cardiovascular risk, either for the management of modifiable cardiac risk factors or for toxicity screening following therapeutic or experimental pharmacological administration. Although it might be possible to improve the therapeutic potential of these cells by the upregulation of B2R expression or signaling, the mechanisms responsible for reduced B2R expression and BK responsiveness in cardiovascular disease are still unknown, and their elucidation will be important to better understand how this might be overcome.

In addition to showing a novel role for the kinin–kinogen pathway, the results of Kränkel et al further highlight the importance of NO production for EPC activity. Although PI3K or eNOS inhibitors were not used in their in vivo studies, both agents reduced the EPC migratory response to BK in vitro to a similar degree as did B2R blockade, suggesting that this response was primarily mediated through eNOS activation. This is reminiscent of the results of earlier studies showing a degree of NO dependency for the actions of VEGF, statins, and PPAR-γ agonists; and thus, eNOS may be a common signaling pathway through which all of these various mediators converge. In this regard, it would also be of interest to study whether pretreatment with VEGF or statins would enhance EPC migration in response to BK or whether selection by migration to another agent (ie, VEGF or SDF-1) would result in similar (or greater) enrichment for angiogenic activity.

The findings of Kränkel et al are exciting additions to our emerging understanding of the biology and therapeutic potential of EPCs. The ability to identify and select a more regenerative progenitor cell B2R expression can easily be measured by flow cytometry, this could potentially be evaluated as a marker of cardiovascular risk, either for the management of modifiable cardiac risk factors or for toxicity screening following therapeutic or experimental pharmacological administration. Although it might be possible to improve the therapeutic potential of these cells by the upregulation of B2R expression or signaling, the mechanisms responsible for reduced B2R expression and BK responsiveness in cardiovascular disease are still unknown, and their elucidation will be important to better understand how this might be overcome.

In addition to showing a novel role for the kinin–kinogen pathway, the results of Kränkel et al further highlight the importance of NO production for EPC activity. Although PI3K or eNOS inhibitors were not used in their in vivo studies, both agents reduced the EPC migratory response to BK in vitro to a similar degree as did B2R blockade, suggesting that this response was primarily mediated through eNOS activation. This is reminiscent of the results of earlier studies showing a degree of NO dependency for the actions of VEGF, statins, and PPAR-γ agonists; and thus, eNOS may be a common signaling pathway through which all of these various mediators converge. In this regard, it would also be of interest to study whether pretreatment with VEGF or statins would enhance EPC migration in response to BK or whether selection by migration to another agent (ie, VEGF or SDF-1) would result in similar (or greater) enrichment for angiogenic activity.

The findings of Kränkel et al are exciting additions to our emerging understanding of the biology and therapeutic potential of EPCs. The ability to identify and select a more regenerative progenitor population could improve the efficacy of cell therapy for cardiovascular disease. Moreover, an improved surface marker of progenitor cell function could advance our understanding of cardiac risk factors and provide a better diagnostic tool for clinical risk stratification for our patients. Although the importance of the kinin–kinogen pathway for EPC activity needs to be confirmed in clinically relevant models of cardiovascular disease, the work of Kränkel et al highlights the importance of host factors and cell selection in the optimization of therapeutic strategies, which may be critical for enhancing the potential benefit of cell therapy in future trials.

Sources of Funding
This work was supported by Canadian Institutes of Health Research and the Krembil Foundation. MRW is supported by a Canadian Institutes of Health Research Jessie Boyd and Charles Siver MD/PhD Student Award.

Disclosures
None.

References
"B₂ or not B₂?": Kinin Receptors and Endothelial Progenitor Cell Dysfunction

Michael R. Ward, Jessie Lavoie and Duncan J. Stewart

_Circ Res._ 2008;103:1202-1203
doi: 10.1161/CIRCRESAHA.108.189084

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 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/103/11/1202

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/