UltraRapid Communication

Young Adult Bone Marrow–Derived Endothelial Precursor Cells Restore Aging-Impaired Cardiac Angiogenic Function

Jay M. Edelberg, Lilong Tang, Koichi Hattori, David Lyden, Shahin Rafii

Abstract—Delivery of young bone marrow–derived stem cells offers a novel approach for restoring the impaired senescent cardiac angiogenic function that may underlie the increased morbidity and mortality associated with ischemic heart disease in older individuals. Recently, we reported that alterations in endothelial cells of the aging heart lead to a dysregulation in the cardiac myocyte platelet-derived growth factor (PDGF)-B–induced paracrine pathway, which contributes to impaired cardiac angiogenic function. Based on these results, we hypothesized that cellular restoration of the PDGF pathway by bone marrow–derived endothelial precursor cells (EPCs) could reverse the aging-associated decline in angiogenic activity. In vitro studies revealed that young murine (3-month-old) bone marrow–derived EPCs recapitulated the cardiac myocyte–induced expression of PDGF-B, whereas EPCs from the bone marrow of aging mice (18-month-old) did not express PDGF-B when cultured in the presence of cardiac myocytes. Transplantation of young, but not old, genetically marked syngeneic bone marrow cells into intact, unirradiated aging mice that populated the endogenous senescent murine bone marrow incorporated into the neovasculature of subsequently transplanted syngeneic neonatal myocardium. Moreover, the young bone marrow–derived EPCs restored the senescent host angiogenic PDGF-B induction pathway and cardiac angiogenesis, with graft survival and myocardial activity in the aging murine host (cardiac allograft viability: 3-month-old controls, 8/8; 18-month-old controls, 1/8; 18-month-old donors receiving bone marrow from 3-month-old mice, 15/16; or 18-month-old mice, 0/6; P<0.05). These results may offer a foundation for the development of novel therapies for the prevention and treatment of cardiovascular disease associated with aging. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2002;90:e89-e93.)

Key Words: endothelium ♦ heart ♦ angiogenesis ♦ aging ♦ bone marrow transplantation

Strategies directed at the molecular and cellular changes in the aging cardiac vasculature may reverse the senescent predisposition for cardiovascular pathology. In younger individuals, myocardial ischemia induces the development of a collateral vasculature supply that partially protects the cardiac tissue from subsequent coronary events.1–4 However, angiogenesis and endothelial function are impaired with aging and contribute to the increased severity of cardiovascular disease in the geriatric population (for review, see Weinsaft and Edelberg5). Approaches specifically aimed at restoring senescent vascular dysfunction may improve clinical outcomes of older persons with cardiovascular disease in whom present treatments are less effective as compared with younger individuals.6–8

Recently we demonstrated an aging-associated impairment in cardiac endothelial induction of platelet-derived growth factor (PDGF)-B that underlies the depression in senescent cardiac angiogenic function.9 We speculated that delivery of young endothelial precursor cells (EPCs) may be employed to revitalize the PDGF-B pathway and reverse the decline in senescent cardiac angiogenic function. There is compelling evidence from our laboratory,10,11 as well as others,12–17 demonstrating that bone marrow–derived EPCs are recruited to angiogenic foci in the peripheral vasculature. Moreover, clinically, the impairment in these cells correlates with an increased risk of myocardial infarction,18 suggesting that renewal of young EPC activity by bone marrow transplantation may restore senescent cardiac angiogenic function.

In the present study, we expand the previous findings, demonstrating that transplantation of young bone marrow restores the pathways critical for cardiac angiogenesis in the aging host. Moreover, this approach does not require ablation of the endogenous aging bone marrow and establishes a new concept in the treatment of aging-related diseases.

Materials and Methods

Molecular Studies

In order to determine the potential of bone marrow–derived EPCs to reconstitute cardiac myocyte–induced PDGF-B–mediated angiogenic function in aging mice, bone marrow cells were isolated from 3- and 18-month-old C57B1/6 mice (Harlan Sprague-Dawley, Indianapolis, Ind) and EPCs cultured, as previously described,17 in DMEM supplemented with 10% fetal calf serum and 50 μg/mL
heparin, 100 µg/mL streptomycin, and 500 µg/mL penicillin (all from Sigma), and 10 ng/mL vascular endothelial growth factor, and 5 ng/mL fibroblast growth factor-2 (R&D Systems). The EPC cultures (initiallyPECAM negative) were expanded for 2 passages, confirmed by Di-Ac-LDL uptake and PECAM staining, and then plated into 12-well dishes (10^5 cells/well) (Costar). Cardiac myocytes (E15.5d) were isolated and plated in 12-mm 0.4 µm pore transwells (10^5 cell/transwell) and then were transferred at different time points (0 to 48 hours) into 3- and 18-month-old bone marrow–derived EPCs seeded wells as previously described.19 As controls for myocyte-induced gene expression, cardiac microvascular endothelial cells were isolated from 3- and 18-month-old C57Bl/6 mice by tail vein injection with 3×10^7 cells, n=5. Oligonucleotide primers were employed: murine PDGF-B (forward) 5'-ATGCGGAGTGCAAGACGCG-3', (reverse) 5'-AAGCACCATTTGCGGC-3'; murine PECAM (forward) 5'-GGATCCATGAACCTTCTCGTGTCTGATG-3', (reverse) 5'-TTCTGGCTTGTGCTCTGTTTGGG-3'; murine PECAM (forward) 5'-CAAGCGGTTGGAATGACAC-3', (reverse) 5'-CAGGCTCTGATGAGACAC-3'; murine β-actin (forward) 5'-GTTGGGC-GCTCTTAGGCACCAA-3', (reverse) 5'-CTCTTTTGGATGTCACGCAGATTAC-3'.

Protein samples were isolated from additional EPC transwells cultured in the presence or absence of cardiac myocytes as previously described19 and were applied to Nunc Maxisorb plates (R&D Systems) for 1 hour at room temperature. The samples were then washed with PBS 3 times, followed by blocking with 5% casein in PBS. Polyclonal antibodies to PDGF-B (1:300 dilution sc-7878, Santa Cruz Biotechnology) and PECAM (1:500 dilution 550274, BD Pharmingen) were then employed and developed with peroxidase-labeled donkey polyclonal antibodies to rabbit and rat IgG (1:1000, Jackson Immunoresearch Laboratories) and assayed as previously described.9,19 All studies were performed a minimum of 3 times.

**Bone Marrow Transplantation**

Bone marrow transplantation was performed as previously described.9,19 Briefly, 3- and 18-month-old C57Bl/6 mice, as well as 3-month-old B6.129Sv-Grosa26 (Rosa-26) mice, were euthanized, and tibias and femurs were removed and trimmed of muscle and extraossial tissue. All the cells in the Rosa-26 expressed LacZ; therefore, transplantation of the Rosa-26 bone marrow into the wild-type isogeneic senescent hosts facilitated the identification of the transplanted cells by X-gal staining. The bones were cut proximally and distally, and the bone marrow flushed with 2% bovine serum albumin in IVD. The cellular pellets were washed with PBS and retransplanted in IVD mice. The bone marrow cells were then injected into IVD mice of wild-type 18-month-old host C57Bl/6 mice by tail vein injection with 300 µL of cells (3-month-old C57Bl/6: 10^5 cells, n=6; 18-month-old C57Bl/6, 10^5; n=6; 3-month-old Rosa-26, 10^5, n=6). The survival rates of all mice transplanted with exogenous bone marrow was 100%. Sets of mice receiving Rosa-26 bone marrow were euthanized 14 days after bone marrow transplantation, and the bone marrow was stained for β-galactosidase activity (X-gal, Fischer Scientific).

**Cardiac Allograft Transplant Studies**

To test the physiological actions of bone marrow transplantation and the recruitment of bone marrow–derived EPCs in cardiac angiogenic function, we employed a cardiac allograft model, which allows assessment of strategies that restore cardiac angiogenic potential in aging mice while controlling the age of cardiac tissue being vascularized. In this model, which recapitulates the PDGF-mediated cardiac myocyte-endothelial cell communication and angiogenic induction,19,22,23 neonatal C57Bl/6 (24-hour-old) murine hearts are transplanted into the pinnas of syngeneic host mice. In the syngeneic mouse, the cardiac allografts are not vascularized due to an impairment in PDGF-B induction in the endothelial cells of the aging mice, and exogenous delivery of PDGF-AB specifically restores the angiogenic defect and promotes the engraftment of the transplanted cardiac tissue.9 One week after bone marrow transplantation the mice received pinnal cardiac allografts to assess senescent cardiac angiogenic activity. Allograft viability was scored by pinnal and transplant integrity 1 week after engraftment, as we have previously described.9 Pinnal electrocardiograms were recorded as previously described to further document the viability of the cardiac allografts.9,19 Intact wild-type 3- and 18-month-old mice (n=8 each) served as positive and negative controls, respectively. Seven days after cardiac allograft transplantation, mice receiving Rosa-26 bone marrow were euthanized, and the exogenous cardiac tissue with surrounding pinnal tissue was sectioned and stained for β-galactosidase activity (X-gal, Fischer Scientific), with Willebrand factor (082, Dako), and PDGF-B (sc-7878, Santa Cruz Biotechnology). In addition to demonstrate the role of PDGF in the restoration of senescent cardiac angiogenic function, at the time of cardiac allograft transplantation, sets of older mice transplanted with 10^7 young bone marrow cells were treated with single subcutaneous pinnal injections of antibodies to neutralize the PDGF pathway (10 µg in 20 µL PBS, AB-20-NA, R&D Systems; n=7), or nonimmune rabbit IgG (10 µg in 20 µL PBS, AB-105-C, R&D Systems; n=7) as we have previously described.9 Studies were performed in compliance with the Institutional Animal Care and Use Committee of Weill Medical College of Cornell University.

**Results**

**Young Bone Marrow–Derived Endothelial Precursor Cells Mediate PDGF-B Cardiac Angiogenic Pathways**

We speculated that bone marrow–derived EPCs of young mice may offer a novel means of reversing the impairment in PDGF-B induction that underlies the senescent dysregulation of cardiac angiogenic pathways.9 To this end, we tested the capacity of young bone marrow–derived EPCs to reestablish the cardiac myocyte–PDGF-B endothelial pathways in the aged cardiac angiogenic environment.9,19 In vitro cardiac myocyte coculture assays revealed that PDGF-B expression was dynamically induced in the young, but not older, bone marrow–derived EPCs (Figure 1a), resulting in the subsequent generation of PDGF-B protein (Figure 1b). Moreover, unlike endothelial cells isolated from the murine cardiac microvasculature,9 EPCs isolated from both the young and older bone marrow did not express VEGF, suggesting that restoration of PDGF-B induction by delivery of young bone marrow EPC may mediate the critical upstream molecular pathways required for the restoration of senescent cardiac angiogenic function.

![Figure 1](https://example.com/figure1.png)

**Figure 1. a, Temporal gene expression profiles of bone marrow–derived EPCs (0 to 48 hours) and cardiac microvascular endothelial cells (CMCEs, 0 and 48 hours) isolated from 3- and 18-month-old mice cocultured in transwells with cardiac myocytes. b, PDGF-B/PECAM protein ratio in 3-month-old bone marrow–derived EPCs cultured with cardiac myocytes.**
Transplanted Young Bone Marrow–Derived Endothelial Precursor Cells Home to Senescent Cardiac Angiogenic Foci

Based on the in vitro results, we tested the potential of young bone marrow–derived EPCs to home to sites of cardiac angiogenesis in the aging host. In order to develop an approach that would facilitate the future application of these studies to modulate intact vasculature function, we elected to transplant the bone marrow of young mice into intact, unirradiated older mice. To test ability of the young bone marrow to augment the population of aging murine bone marrow LacZ+/Rosa-26 bone marrow was transplanted intravenously into intact isogenic older mice 1 week before inducing cardiac angiogenesis. Analysis of these mice revealed that the genetically marked bone marrow (β-galactosidase–positive) cells were recruited to and engrafted in the senescent endothelium.9 Based on the ability of the young bone marrow–derived EPCs to reconstitute PDGF-B induc- tion in the presence of cardiac myocytes and their recruitment to cardiac angiogenic foci, we hypothesized that these cells could rescue cardiac angiogenic function in the aging mice. Remarkably, transplantation of bone marrow of 3-month-old mice into intact 18-month-old murine hosts maintained the viability and restored the function of the exogenous cardiac tissue (Figure 3a). Transplantation with the bone marrow of 18-month-old mice failed to reverse the aging-associated decline in cardiac angiogenic function (Figure 3b). The restoration of the senescent vascular function by the young bone marrow cells demonstrated a cellular dose-dependent response. Moreover the angiogenic response in the bone marrow–transplanted aging mice was specifically blocked by inhibition of PDGF (Figure 3b), similar to results observed in young intact hosts in which neutralization of PDGF pathways prevented vascularization of cardiac tissue but not pulmonary allografts,9 thus confirming the importance of PDGF in the young bone marrow EPC-mediated restoration of senescent cardiac angiogenic function in vivo.

Discussion

The present studies demonstrate that reconstitution of bone marrow–derived EPC function can specifically promote cardiac angiogenic function in the aging host. Young bone marrow–derived EPCs are capable of populating the intact, senescent bone marrow, homing to sites of cardiac angiogenic induction, and restoring pathways required for vascular function. Our findings represent a significant advance in defining the mechanistic roles of bone marrow–derived EPCs in the peripheral vasculature. Although previous studies have demonstrated the incorporation of bone marrow–derived EPCs into the peripheral vascular, these studies did not elucidate the relevant physiological contribution of the exogenous endothelium in angiogenic function.10,15,25,26 Recent studies have revealed that direct injection of bone marrow cells into cardiac tissue promotes vascular activity26,27; however, the molecular mechanisms of the specific cellular mediators of this function remain to be defined. Our results demonstrate that bone marrow–derived EPCs recruited from the trans-
planted young bone marrow cells restore PDGF-mediated autocrine as well as other potentially critical autocrine and paracrine pathways required for cardiac angiogenic function. Moreover, the present studies were performed in unirradiated, wild-type aged mice, demonstrating the potential utility of transplanting unfractionated or specific linages of young bone marrow cells without ablating the host bone marrow to restore and/or augment the function of critical cell populations in the senescent bone marrow.

Transplantation of young bone marrow cells that give rise to EPC populations offers a novel means of delivering angiogenic function in the aging host and may have a therapeutic role in reversing the aging-associated impairment in endothelial function, as well as modulating hemostatic and rheologic changes in the senescent vascular. Overall, restoration of bone marrow–derived EPC function offers a foundation for the development of strategies specifically tailored for the treatment of cardiovascular disease in older individuals.

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References

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