Marrow-Derived Stromal Cells Express Genes Encoding a Broad Spectrum of Arteriogenic Cytokines and Promote In Vitro and In Vivo Arteriogenesis Through Paracrine Mechanisms

T. Kinnaird, E. Stabile, M.S. Burnett, C.W. Lee, S. Barr, S. Fuchs, S.E. Epstein

Abstract—We recently demonstrated that marrow stromal cells (MSCs) augment collateral remodeling through release of several cytokines such as VEGF and bFGF rather than via cell incorporation into new or remodeling vessels. The present study was designed to characterize the full spectrum of cytokine genes expressed by MSCs and to further examine the role of paracrine mechanisms that underpin their therapeutic potential. Normal human MSCs were cultured under normoxic or hypoxic conditions for 72 hours. The gene expression profile of the cells was determined using Affymetrix GeneChips representing 12,000 genes. A wide array of arteriogenic cytokine genes were expressed at baseline, and several were induced 1.5-fold by hypoxic stress. The gene array data were confirmed using ELISA assays and immunoblotting of the MSC conditioned media (MSCCM). MSCCM promoted in vitro proliferation and migration of endothelial cells in a dose-dependent manner; anti-VEGF and anti-FGF antibodies only partially attenuated these effects. Similarly, MSCCM promoted smooth muscle cell proliferation and migration in a dose-dependent manner. Using a murine hindlimb ischemia model, murine MSCCM enhanced collateral flow recovery and remodeling, improved limb function, reduced the incidence of autoamputation, and attenuated muscle atrophy compared with control media. These data indicate that paracrine signaling is an important mediator of bone marrow cell therapy in tissue ischemia, and that cell incorporation into vessels is not a prerequisite for their effects. (Circ Res. 2004;94:678-685.)

Key Words: marrow stromal cells • arteriogenesis • bone marrow cells • cytokines

An important compensatory response to atherosclerotic obstructive arterial disease is collateral development, a complex process requiring that multiple genes coordinately express their products in an appropriate time-dependent manner. However, the natural capacity of collaterals to remodel and enlarge to compensate for the reduced flow that occurs after occlusion of a major artery is rarely sufficient to restore maximal flow capacity to levels required under various stress-conditions.

Although several protein and gene-based strategies have succeeded in enhancing collateral development in animal models of ischemia, clinical studies thus far have been disappointing. Given that the natural response to tissue ischemia is such a complex process, the delivery of a single growth factor may be too simple an approach. Thus, a great deal of interest has arisen in the potential of cell-based strategies in augmenting collateral responses, and several groups have demonstrated incorporation of various bone marrow–derived cells into new or remodeling vessels.

However, the actual magnitude of incorporation of bone marrow–derived cells into vascular structures varies substantially between studies. Although some studies report over 50% of capillaries containing transplanted cells, other studies have reported only occasional positive vessels despite impressive improvement in perfusion. Taken together, these data suggest that other mechanisms apart from cell incorporation may contribute to collateral remodeling observed after bone marrow–derived cell therapy in various models of ischemia. Furthermore, we recently demonstrated that marrow stromal cells (MSCs) augment collateral remodeling through release of several cytokines such as VEGF and bFGF rather than via cell incorporation into new or remodeling vessels. Therefore, the purpose of the present study is to characterize the full spectrum of cytokine genes expressed by MSCs and to further examine the role of paracrine mechanisms that underpin the biological effects of MSC therapy for tissue ischemia.

Materials and Methods

Human Cell Lines

Normal human MSCs (derived from a single 19-year-old healthy donor) were purchased from Clonetics (Walkersville, Md) and cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 1% penicillin-streptomycin (DM-10). These cells were previously demonstrated to...
be negative for CD34 and CD45 surface markers, and positive for CD44, CD105, and CD166. Passages 3 to 5 were used for in vitro experiments. Human umbilical vein endothelial cells (ECs) were purchased from American Type Culture Collection (ATCC, Manassas, Va) and cultured in endothelial growth media-2 (EGM-2; Clonetics). Human aortic smooth muscle cells (SMCs) were purchased from ATCC and cultured in Medium-199 supplemented with 10% fetal bovine medium and 1% penicillin-streptomycin (M-10). Passages 3 to 8 were used for in vitro experiments. Cells were cultured in 20% O2 and 5% CO2 during normoxia experiments, and in 1% O2 and 5% CO2 using a hypoxia chamber for hypoxia experiments.

RNA Preparation
Total RNA was extracted from normoxia and hypoxia exposed (72 hours) human MSCs (2 plates per analysis) using TRIzol Reagent (Invitrogen) according to the manufacturer’s instructions. RNA was cleaned using a RNeasy mini kit (Qiagen) and stored at −80°C.

Microarray Analysis
Double-stranded cDNA was synthesized from 8 µg of total RNA. For the first cDNA strand synthesis, oligo(dt) primers were annealed to the RNA and extension by reverse transcriptase was performed in the presence of deoxynucleotides. The second strand was synthesized using DNA polymerase I and purified using a phase lock gels-phenol/chloroform extraction, followed by ethanol precipitation. In vitro transcription, using double-stranded cDNA as a template in the presence of biotin-labeled ribonucleotides, was performed by using an Enzo in vitro transcription kit (Enzo Diagnostics). Biotin-labeled cRNA was purified, fragmented, and hybridized to Affymetrix Human Genome U133A GeneChips (Affymetrix, Santa Clara, Calif). Hybridization, washing, antibody amplification, staining, and scanning of probe arrays were performed according to the Affymetrix Technical Manual. Scanned raw data were processed with Affymetrix GeneChip v 5.1 software. A hypoxia fold induction of >1.5 was considered significant.

Human Conditioned Media Collection, Preparation, and Analysis
For ELISA, human MSC-conditioned media (hMSCCM) was collected after 24 hours of culture, centrifuged at 2000 rpm for 10 minutes, and passed through a 0.3 µm filter. The concentration of MSCCM cytokines was measured using sandwich ELISA kits (VEGF, bFGF, MCP-1, and PlGF; R&D systems). After media collection, cells were lysed and total protein counted with the MicroBCA protein assay (Pierce). ELISA values were corrected for total cell protein. EC- and SMC migration assays were performed using Transwell culture chambers (Costar, Corning). Cells were suspended in DMEM supplemented with 2% serum and placed in the top chamber. For EC proliferation, the media was replaced with varying dilutions of MSCCM, EGM-2 (positive control), recombinant VEGF 4 ng/mL (positive control, R&D Systems), DM-10 (normal control), or boiled hMSCCM (negative control). Further DM-10 samples were supplemented with recombinant VEGF to concentrations coinciding with the concentration of VEGF present in the conditioned medium. To examine the role of cytokines in isolation, 10 µg/mL anti-VEGF antibody (Sigma), 5 µg/mL anti-FGF antibody (Sigma), or 5 µg/mL anti-PDGF antibody (Sigma) was added to hMSCCM dilutions in additional wells. Further DM-10 samples were also supplemented with recombinant VEGF to concentrations found in the relevant dilution of hMSCCM. For SMC proliferation, the media was replaced with varying dilutions of MSCCM, PDGF (10 ng/mL, positive control, Clonetics), or DM-10 (normal control). Cultures were continued for 72 hours, after which the cells were recovered and counted using a Coulter counter. Data are reported as the mean percentage change in proliferation when compared with control media (DM-10).

Cell Migration Assay
EC and SMC migration assays were performed using Transwell culture chambers (Costar, Corning). Cells were suspended in DMEM supplemented with 2% serum and placed in the top chamber (4×10⁵/well). For EC migration, DM-10 (normal control), hMSCCM, boiled hMSCCM, and VEGF (4 ng/mL, positive control) were added to the lower chamber. For SMC migration, DM-10, hMSCCM, boiled hMSCCM, and PDGF (10 ng/mL, positive control) were added to the lower chamber. Cells were incubated overnight, and the top layer of the membrane scraped gently to remove any cells. Cells on the lower surface of the membrane were stained using Hema-3 staining kit (Biochemical Sciences). Six random fields per membrane were counted. Data are reported as the mean percentage or fold change in proliferation when compared with control media (DM-10).

Murine MSC Preparation and Culture
Murine bone marrow was harvested by flushing the tibiae and femurs of Balb/C mice (two mice per culture; Jackson Laboratories, Bar Harbor, Maine) with DM-10. The pooled marrow was dispersed, plated in DM-10, and cultured for 72 hours. Nonadherent cells were washed off and adherent cells expanded until confluent (~7 to 10 days). FACs analysis of up to passage 6 demonstrated persistence of lymphohematopoietic cells (CD34+ or CD45+ or both). Therefore, MSCs were purified from the heterogeneous cultured cells. The CD34+/CD45− fraction was isolated by labeling with FITC-conjugated anti-CD34 antibody (PharMingen) followed by simultaneous incubation with a cocktail of anti-FITC and anti-CD45 magnetic beads (Miltenyi Biotech). Cells were passed through a magnetic column, the double-negative fraction collected, and replated. Repeat FACs analysis was performed and demonstrated that cells did not express CD31, CD34, CD45, and CD117, and expressed high levels of CD44, CD90, and CD105 (data not shown) typical of marrow-derived stromal cells, and in keeping with previous published data. For in vivo experiments, murine MSCCM (mMSCCM) was collected after 72 hours and then concentrated 2-fold using Microcon YM-10 centrifugal filters (Amicon). As a control, DM-10 was filtered and concentrated in a likewise fashion.

Animal Surgery and Murine MSCCM injection
All animal interventions were approved by the Animal Care and Use Committee of the MedStar Research Institute. Under narcosis, 12-week-old Balb/C mice (n=5 per group) were subjected to operative intervention to create unilateral hindlimb ischemia. The right femoral artery was exposed in the mid thigh, dissected from the femoral vein and nerve, and then ligated just proximal to the popliteal bifurcation. In preliminary studies, MSCCM injection immediately after femoral ligation failed to improve flow recovery. Therefore, in the present study, mMSCCM injection was delayed by 24 hours to allow the mice to partially recover from the surgical insult. A total of 50 µL of mMSCCM or DM-10 was injected into the adductor muscle at four sites adjacent to and within 1 mm of the ligation site. The injections were repeated at 48 hours and 72 hours.

Perfusion Imaging
Laser doppler perfusion imaging (LDPPI) (Moor Instruments) was utilized to record serial blood flow measurements. For consistent measurements, imaging was performed after limb hair was removed, and after mice had been placed on a heating plate at 37°C to minimize temperature variation. Calculated perfusion is expressed as a ratio of the ischemic to normal limb. Previous data have
suggested a close linear relationship between recovery of perfusion as assessed by LDPI, and positive remodeling of adductor collateral vessels.\textsuperscript{14}

### In Vivo Assessment of Limb Function

Functional assessment of the ischemic limb was performed using a modification of a clinical standard score.\textsuperscript{15} A semiquantitative assessment of ambulatory impairment of the ischemic limb was performed serially (0=no dragging but no plantar flexion, 1=plantar flexion, 2=no dragging but no plantar flexion, 3=dragging of foot). A blinded observer assigned all scores.

### Histological Assessment of Collateral Morphology

After completing blood flow assessment, sections of adductor muscles were stained with van Gieson’s solution. Only arteries, identified by the presence of a continuous internal elastic laminae and muscle spindles, and with a mathematically derived area $>300$ μm$^2$, were counted. Total cross sectional area was calculated using Image-Pro software, with the smallest internal luminal distance measured as the radius.

### Data and Statistical Analysis

Expression analysis data were verified by performing experiments in duplicate. All ELISA, immunoblotting, and cell studies were performed at least in triplicate. All results are presented as mean±SEM. Statistical significance was evaluated using an unpaired student $t$ test, or ANOVA where indicated. A value of $P<0.05$ was considered significant.

### Results

#### Gene Array of Human MSCs

MSCs expressed genes for a wide array of arteriogenic cytokines (Table). The expression of FGF-2, FGF-7, interleukin-1 and interleukin-6, placental growth factor, TGF-β, TNF-α, and VEGF-A were all augmented by exposure to hypoxic stress.

#### Cytokine Release From MSCs

To complement gene expression patterns, we analyzed the cytokine content of hMSC\textsuperscript{CM} using ELISA (Figure 1). Baseline and hypoxic augmentation of VEGF-A secretion (375 pg/mg protein in normoxia versus 698 pg/mg in hypoxia; $P<0.01$) was confirmed with a similar pattern also seen for FGF-2 (2320 pg/mg versus 3970 pg/mg; $P<0.05$), and interleukin-6 (3885 pg/mg versus 7665 pg/mg; $P<0.01$), reflecting similar changes in gene expression. Although placental growth factor gene expression was augmented 3-fold by hypoxic stress, secretion of the cytokine was not significantly altered (119 pg/mg versus 164 pg; $P=NS$). Monocyte chemoattractant protein-1 gene expression was unchanged after exposure to hypoxic stress and a similar pattern of secretion was also seen (150 pg/mg versus 70 pg/mg; $P=NS$). Immunoblotting of the MSC\textsuperscript{CM} for angiopoietin-1, PDGF, metalloproteinase-9, and plasminogen activator also demonstrated similar cytokine release profiles to the expression profiles (Figure 2). ECs released minimal cytokines under baseline conditions and in response to hypoxia.

#### hMSC\textsuperscript{CM} Effect on Endothelial Cell Proliferation and Migration

To examine whether hMSC\textsuperscript{CM} exhibited biological effects relevant to collateral remodeling, a series of EC proliferation assays were performed. hMSC\textsuperscript{CM} significantly enhanced EC

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### Table: Marrow-Derived Stromal Cells Proangiogenic/Proarteriogenic Gene Expression

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Angiogenic/Arteriogenic Function</th>
<th>Fold Induction With Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiopoietin-1</td>
<td>EC migration, vessel stabilization</td>
<td>...</td>
</tr>
<tr>
<td>Fibroblast growth factor-2</td>
<td>EC and SMC proliferation and migration</td>
<td>1.62</td>
</tr>
<tr>
<td>Fibroblast growth factor-7</td>
<td>EC proliferation and stabilization</td>
<td>1.82</td>
</tr>
<tr>
<td>Hepatoma growth factor</td>
<td>SMC proliferation</td>
<td>...</td>
</tr>
<tr>
<td>Interleukin-1</td>
<td>VEGF induction</td>
<td>1.91</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>VEGF induction</td>
<td>2.26</td>
</tr>
<tr>
<td>Metalloproteinase-1</td>
<td>Loosens matrix, tubule formation</td>
<td>...</td>
</tr>
<tr>
<td>Metalloproteinase-2</td>
<td>Loosens matrix, tubule formation</td>
<td>...</td>
</tr>
<tr>
<td>Metalloproteinase-9</td>
<td>Loosens matrix</td>
<td>...</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Monocyte migration</td>
<td>...</td>
</tr>
<tr>
<td>M-CSF</td>
<td>Monocyte proliferation/migration</td>
<td>...</td>
</tr>
<tr>
<td>Plasminogen activator</td>
<td>Degradation matrix molecules</td>
<td>...</td>
</tr>
<tr>
<td>Platelet-derived growth factor</td>
<td>SMC proliferation and migration</td>
<td>...</td>
</tr>
<tr>
<td>Stem cell–derived factor</td>
<td>Progenitor cell homing</td>
<td>...</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>Vessel maturation, EC proliferation</td>
<td>2.11</td>
</tr>
<tr>
<td>Tumor necrosis factor-α</td>
<td>Degradation matrix molecules, EC proliferation</td>
<td>1.69</td>
</tr>
<tr>
<td>VEGF-A</td>
<td>EC proliferation, migration, tube formation</td>
<td>2.47</td>
</tr>
<tr>
<td>VEGF-B</td>
<td>EC proliferation, migration, tube formation</td>
<td>...</td>
</tr>
</tbody>
</table>

MCP-1 indicates monocyte chemoattractant protein-1; M-CSF, macrophage-specific colony-stimulating factor; VEGF, vascular endothelial growth factor; EC, endothelial cell; and SMC, smooth muscle cell.
proliferation over control and was comparable to growth seen with EGM-2 (Figure 3A). The proliferative effect of hMSC was completely abolished by boiling, suggesting these effects were due to a specific receptor/ligand interaction. As expected, the mitogenic effect of recombinant VEGF was inhibited by the addition of VEGF-blocking antibody (Figure 3A). In contrast to this, addition of the same antibody to hMSC only partially attenuated EC proliferation (5.5-fold without antibody versus 3.6-fold with antibody; *P<0.001; Figure 3B). Similarly, addition of an FGF-2 blocking antibody only partly attenuated proliferation (5.5-fold versus 4.6-fold, respectively; *P<0.05, †P<0.001 versus control media). The addition of both blocking antibodies significantly reduced the mitogenic effects, although the effect was still significant over control (*P<0.001). There was no significant change in EC proliferation in response to hMSC following the addition of anti-PDGF antibody. Additionally, reconstitution of DM-10 with recombinant VEGF to concentrations seen in the hMSC dilutions failed to stimulate EC proliferation to the same extent as hMSC (Figure 3C). The data strongly suggest that the mitogenic effects of hMSC are due to multiple cytokines. Finally, a hMSC dose-response relationship was also demonstrated (Figure 3C).

To further examine the biological effects of hMSC, its effect on EC migration was studied. hMSC induced a 5.5-fold increase in EC migration compared with control, although this did not achieve the fold increase observed with recombinant VEGF (Figures 4A and 4B). As with EC proliferation, boiling eliminated the chemoattractant properties of hMSC.

**hMSC Effect on Smooth Muscle Cell Proliferation and Migration**

hMSC stimulated proliferation of SMCs in a dose-responsive manner, although this did not reach the effect seen with recombinant PDGF-β. As in EC cultures, the SMC proliferative effect of hMSC was abolished by boiling (Figure 5A). hMSC also exerted a chemoattractant effect on SMCs, although the effect was only weak in comparison to PDGF-β and was inhibited by boiling (Figure 5B).

**Hindlimb Blood Flow, Limb Recovery, and Collateral Morphology After mMSC Injection**

Having established that MSCs secrete many arteriogenic cytokines, and that the hMSC exerts in vitro biological effects relevant to collateral remodeling, we proceeded to examine whether, as part of their therapeutic benefit, MSCs were able to contribute to collateral remodeling through paracrine mechanisms. To do this, we injected mMSC directly into the adductor muscle (area of collateral remodeling) in a mouse model of hindlimb ischemia. In mice receiving control media, flow returned to ≈50% of the nonischemic limb by day 28. In contrast, in those mice receiving mMSC there was a significant improvement in flow (Figure 6A) by day 3, which was maintained for the duration of the study (*P<0.05 by ANOVA). Representative flow images are displayed in Figure 6B. In mice receiving MSC, total arterial cross sectional area was significantly
increased in those mice compared with control (8380 μm² versus 4303 μm²; \( P < 0.05 \); Figure 6C).

The improved flow recovery was associated with improved hindlimb appearance and function. Mice receiving control media experienced severe ischemic damage resulting in a 60% incidence of autoamputation by day 28. However, mice receiving mMSCCM displayed less ischemic damage with a 20% autoamputation rate. Similarly, in mice receiving mMSCCM, limb function was significantly better than those mice receiving control media (ambulatory score 2.25 versus 1.25, respectively; \( P < 0.05 \); Figure 6D).

Improved flow recovery also attenuated the calf muscle atrophy observed after femoral artery ligation. In control mice, muscle loss was significantly greater than in those mice receiving mMSCCM (69% versus 41%, respectively; \( P < 0.05 \); Figure 6E).

**Discussion**

Cells of the marrow stroma maintain hematopoietic stem cells and their progeny through a variety of molecular mechanisms including direct cell-to-cell interactions and, importantly, through local release of supportive cytokines.\(^{16,17}\) Isolated reports have previously demonstrated release of VEGF and bFGF, but the present study is the first to our knowledge to definitively characterize the full spectrum of arteriogenic cytokines released by marrow-derived stromal cells. In addition, previous studies have documented marrow stromal cell secretion of hepatocyte growth factor,\(^{18}\) insulin-like growth factor,\(^{19}\) and MCP-2/MCP-3,\(^{20}\) although mRNA for these cytokines was not found in the present study. Importantly, hypoxia also led to increases in the mRNA expression and secretion of several important cytokines such as VEGF and FGF-2 without adversely affecting release of any other cytokines. This is of relevance as the milieu into which cells are injected, such as ischemia versus nonischemia, is likely to have a major influence on their subsequent behavior.

The ability of bone marrow cells to secrete multiple arteriogenic cytokines has led to several studies demonstrating these cells enhance collateral flow, and the responsible mechanism has often been ascribed to these cells incorporating into the developing collaterals. However, the actual magnitude of incorporation of bone marrow–derived cells into vascular structures varies substantially among studies. Although some studies report over 50% of capillaries con-
taining transplanted cells, other studies have reported only occasional positive vessels despite noting impressive improvements in perfusion.8–10 Taken together, these data suggest that other mechanisms apart from cell incorporation contribute to collateral remodeling observed after bone marrow–derived cell therapy in various models of ischemia.

The present study demonstrates that numerous arteriogenic cytokines are released by MSCs and, importantly, that injection of cells themselves is not required for therapeutic benefit, but that the release of such cytokines is sufficient to mediate arteriogenesis and enhance collateral flow. However, it is likely that complimentary mechanisms may contribute to the beneficial effects on blood vessel formation seen after cell therapy. Marrow stromal cells—also termed mesenchymal stem cells—have been demonstrated to differentiate into smooth muscle and endothelial cell lineages,21–24 and thus may contribute cells directly to new or remodeling vessels. Nonetheless, the importance of the mechanism is still controversial.

Cytokines have not only individual effects, but one cytokine may potentiate (or inhibit) the effect of another. A synergistic relationship between VEGF and bFGF was reported in a rabbit ischemic hindlimb model, and placental growth factor appears to potentiate the effects of VEGF, both in vitro and in vivo models.25,26 Other studies have demonstrated synergism between PDGF and FGF-2 as well as between angiopoietin-1 and VEGF.27,28 The present study also demonstrates this synergism. Blocking the effects of VEGF and bFGF in MSCCM only partly attenuates the mitogenic effects of the MSCCM on endothelial cells. Reconstitution of control media with recombinant VEGF to similar levels as that found in the MSCCM stimulates endothelial cell proliferation, but not nearly to the extent as achieved with whole MSCCM. Taken together, these data imply that multiple cytokines secreted by MSCs have additive or synergistic effects on cell proliferation, and as such MSC therapy may be more effective than single protein approaches in augmenting...
tissue perfusion. It is also interesting to speculate that MSCs could be used therapeutically rather than MSCs themselves, thus avoiding many practical issues regarding cell therapy.

Previous work examining the role of MSCs in angiogenesis demonstrated, using a Matrigel implantation model, that MSCs could augment capillary in-growth through paracrine mechanisms. In that study, MSCs out to passage 14 were used, and their effects could be completely inhibited by addition of neutralizing anti-VEGF antibodies. These observations contrast with the present results. However, we have observed a gradual decrease over time in the release of PlGF and bFGF in MSC cultures (data not shown), whereas VEGF and MCP-1 levels remain relatively constant up to 4-weeks. Thus changes in the cytokine release profile over time may explain differences between this and previous studies. In the clinical setting, therefore, the timing of cell harvest may have important consequences for cell therapy in patients.

Given the importance of paracrine signaling in MSC/hematopoietic cell interactions, it is perhaps not surprising that MSC can augment collateral remodeling through paracrine mechanisms. However, previous studies have suggested this phenomenon may not be restricted to MSCs and that other bone marrow–derived cells may also influence blood flow recovery through release of arteriogenic cytokines. For example, bone marrow mononuclear cells contain mRNA for VEGF, bFGF, and angiopoietin-1, and after injection of BM media alone enhances collateral perfusion and remodeling in a murine model of hindlimb ischemia, reducing tissue atrophy and migration. These effects are dose-dependent and in vitro stimulate endothelial and smooth muscle cells to proliferate, suggesting that these angioblasts may be a source of proangiogenic factors. Endothelial progenitor cells in vitro also release several relevant cytokines, including VEGF and GM-CSF. Thus, previous data in combination with the present study imply that bone marrow–derived progenitor cells can improve tissue ischemia in part through paracrine mechanisms. However, the exact degree to which this occurs is likely to vary from cell-to-cell and from milieu-to-milieu.

In summary, our data demonstrate that marrow-derived stromal cells secrete a broad spectrum of cytokines, which in vitro stimulate endothelial and smooth muscle cells to proliferate and migrate. These effects are dose-dependent and appear to be mediated by several cytokines. Furthermore, local injection of marrow stromal cell–derived conditioned media alone enhances collateral perfusion and remodeling in a murine model of hindlimb ischemia, reducing tissue atrophy and limb damage, and improving limb function, suggesting that paracrine signaling is an important mediator of bone marrow cell therapy in tissue ischemia.

Acknowledgments

This work was funded by an internal grant from the Cardiovascular Research Institute, Washington Hospital Center, Washington, DC.

References


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_Circ Res._ 2004;94:678-685; originally published online January 22, 2004; doi: 10.1161/01.RES.0000118601.37875.AC

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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In an article by Kinnaird et al (Circ Res. 2004;94:678–685), the authors made an error in the stated number of animals assessed and in the calculated percentage of animals that underwent auto-amputation. The authors incorrectly stated: “Under narcosis, 12-week-old Balb/C mice (n = 5 per group) . . . ” The correct number of animals is 4 per group.

The authors also incorrectly stated: “Mice receiving control media experienced severe ischemic damage resulting in a 60% incidence of auto-amputation by day 28. However, mice receiving mMSCs displayed less ischemic damage with a 20% auto-amputation rate.” The correct numbers are 50% auto-amputation rate in the control group and 0% rate in the media group.