

Augmentation of Postnatal Neovascularization With Autologous Bone Marrow Transplantation

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Background—Endothelial progenitor cells (EPCs) have been identified in adult human peripheral blood. Because circulating EPCs should originate from bone marrow (BM), we examined whether BM mononuclear cells (BM-MNCs) can give rise to functional EPCs and whether transplantation of autologous BM-MNCs might augment angiogenesis and collateral vessel formation in a rabbit model of hindlimb ischemia.

Methods and Results—Rabbit BM-MNCs were isolated by centrifugation through a Histopaque density gradient and cultured on fibronectin. EPCs developed from BM-MNCs in vitro, as assessed by acetylated LDL incorporation, nitric oxide (NO) release, and expression of von Willebrand factor and lectin binding. Unilateral hindlimb ischemia was surgically induced in rabbits (n=8), and fluorescence-labeled autologous BM-MNCs were transplanted into the ischemic tissues. Two weeks after transplantation, fluorescence microscopy revealed that transplanted cells were incorporated into the capillary network among preserved skeletal myocytes. In contrast, transplanted autologous BM-fibroblasts did not participate in EC capillary network formation (n=5). Then, in an additional 27 rabbits, saline (control; n=8), autologous BM-MNCs (n=13; $6.9 \pm 2.2 \times 10^6$ cells/animal), or BM-fibroblasts (n=6; $6.5 \pm 1.5 \times 10^6$ cells/animal) were injected into the ischemic tissues at postoperative day 7. Four weeks after transplantation, the BM-MNC-transplanted group had more angiographically detectable collateral vessels (angiographic score: 1.5 ± 0.34 versus 0.94 ± 0.26 and 1.1 ± 0.14 ; $P < 0.05$), a higher capillary density (23 ± 5.8 versus 10 ± 1.9 and 11 ± 0.8 per field; $P < 0.001$), and a greater laser Doppler blood perfusion index (505 ± 155 versus 361 ± 35 and 358 ± 22 U; $P < 0.05$) than the control and BM-fibroblast-transplanted groups.

Conclusions—Direct local transplantation of autologous BM-MNCs seems to be a useful strategy for therapeutic neovascularization in ischemic tissues in adults, consistent with “therapeutic vasculogenesis.” (*Circulation*. 2001;103:897-903.)

Key Words: angiogenesis ■ bone marrow ■ transplantation ■ cells ■ ischemia