



Determination of bone marrow–derived endothelial progenitor cell significance in angiogenic growth factor–induced neovascularization in vivo

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(Received 7 January 2002; revised 18 April 2002; accepted 23 April 2002)

Objective. Our laboratory and others recently provided evidence indicating that endothelial progenitor cells (EPCs) participate in postnatal neovascularization. However, the extent to which EPCs contribute to adult neovascularization remains unclear. To address this issue, we investigated the quantitative contribution of EPCs to newly formed vascular structures in an in vivo Matrigel plug assay and corneal micropocket assay.

Materials and Methods. Lethally irradiated FVB mice were transplanted with bone marrow (BM) mononuclear cells from transgenic mice constitutively expressing β -galactosidase (β -gal) encoded by the *lacZ* gene regulated by an endothelial-specific *tie-2* promoter. Reconstitution of the transplanted BM leads to the expression of *lacZ* in mice, which is restricted to BM cells expressing *tie-2*.

Results. Four weeks after BM transplantation (BMT), *tie-2/lacZ/BMT* mice were implanted with either Matrigel containing fibroblast growth factor-2 subcutaneously or with a vascular endothelial growth factor pellet into the cornea. After 7 days, the Matrigel plug or the cornea was removed and analyzed by X-gal staining or immunostaining for β -gal. X-gal staining of the Matrigel plug identified $5.7\% \pm 1.2\%$ of endothelial cells (ECs) as cells originated from BM-derived EPCs, whereas the more sensitive technique of immunofluorescence identified $26.5\% \pm 0.9\%$ of ECs. Similarly, EPC-derived cells comprised $5.0\% \pm 2.4\%$ and $17.7\% \pm 3.6\%$ of the ECs in corneal neovascularization identified by X-gal staining and immunohistochemistry, respectively. Ki67 staining of the corneal tissue documented that the majority of EPC-derived cells were actively proliferating in situ.

Conclusion. These findings suggest that BM-derived EPCs make a significant contribution to angiogenic growth factor–induced neovascularization that may account for up to 26% of all ECs. © 2002 International Society for Experimental Hematology. Published by Elsevier Science Inc.
