Contribution of endogenous endothelial progenitor cells to neovascularization and astrogliosis in spinal cord injury

INTRODUCTION:
Spinal cord injury causes initial mechanical damage, followed by ischemia-induced, secondary degeneration deteriorating the tissue damage. While endothelial progenitor cells (EPCs) have been reported to play an important role for pathophysiological neovascularization in various ischemic tissues, the EPC kinetics following spinal cord injury has never been elucidated. In this study, we therefore assessed the in vivo kinetics of bone marrow-derived EPCs by EPC colony forming assay and Bone marrow transplantation (BMT) from Tie2/lacZ transgenic mice into wild type mice with spinal cord injury.

METHODS
Bone Marrow Transplantation (BMT) model with Tie2/lacZ transgenic mice: Bone marrow mononuclear cells (MNCs) were collected from Tie2/lacZ transgenic mice, which constitutively express β-galactosidase (β-gal) encoded by lacZ under the transcriptional regulation of an endothelium-specific promoter, Tie2. Wild mice (C57BL/6, 6 weeks old) were lethally irradiated with 12 Gy and received intravenous infusion of 2×10^6 BM-MNCs from Tie2/lacZ transgenic mice. At 4 weeks post-BMT, by which time the bone marrow of the recipient mice was reconstituted, surgical operation was performed to induce spinal cord injury.

Mouse Spinal Cord Injury Models: After laminectomy at the 10th thoracic spinal vertebrae, spinal cord crush injury was performed by compressing the cord laterally from both sides with number 5 Dumont forceps for 10 seconds.

EPC colony forming assay using peripheral blood MNCs: To assess the endothelial commitment and differentiation capacity, an EPC colony forming assay (EPC-CFA) established in our laboratory was performed using circulating MNCs. Peripheral blood (0.6–1.0 ml/mouse) was collected from non-injured mice (pre SCI) and injured ones at days 0 (immediately after SCI), 3, 7 and 14 after spinal cord injury. The EPC-CFA was performed by culturing 1×10^6 PB-MNCs in methyl cellulose-(immediately after SCI), 3, 7 and 14 after spinal cord injury. The EPC-collected from non-injured mice (pre SCI) and injured ones using circulating MNCs. Peripheral blood (0.6~1.0 ml/ mouse) was forming assay (EPC-CFA) established in our laboratory was performed

RESULTS
Serial change in EPC colony formation from PB-MNCs
PB-MNCs slightly decreased immediately after SCI, however gradually increased at day 3 or later. The number of PB-MNCs was significantly greater at days 7 and 14 post injury than pre injury. The vasculogenic potential of PB-MNCs was serially assessed by EPC-CFA. The number of primitive, definitive and total EPC-CFUs peaked at day 3 after SCI and then gradually decreased at days 14 and 28 (Figure 1). These results suggest that spinal cord injury may augment mobilization and differentiation capacity of BM-derived and circulating EPCs, possibly for the spinal cord tissue repair.

DISCUSSION
Our findings in this study suggest that bone marrow-derived EPCs may contribute to the tissue repair by augmenting neovascularization and astrogliosis following spinal cord injury.