Introduction

Recovery in central nervous system disorders is hindered by the limited ability of the vertebrate central nervous system to regenerate lost cells, replace damaged myelin, and re-establish functional neural connections. Cell transplantation to repair central nervous system disorder is a vibrant area of research with the goal of reducing functional deficit. Recent evidence demonstrates that bone marrow contains hematopoietic stem cells producing all the blood cells and mesenchymal stem cells. Moreover, transplantation of hematopoietic stem cells has generated unexpected phenotypes in vivo, including muscle cells, liver cells, brain cells (1,2) and others. In the present study, we employed a mouse model of spinal cord injury and transplanted hematopoietic stem cells from bone marrow into the injured spinal cords. Based on the results, we discuss a possible role of the transplantation on their functional recovery.

Materials and Methods

Purification of hematopoietic stem cell fraction: We collected total bone marrow cells from femurs of male Rosa26 mice. The mice were pretreated and overexpressed β-galactosidase ubiquitously. The cells were analyzed by FACS Vantage (Becton Dickinson, Tokyo, Japan) and e-kit' Sca-1' Lin cells were sorted, yielding primitive hematopoietic stem cells (3). Model of spinal cord injury and cell transplantation: Female C57Bl/6 mice were laminectomized at the T8 level under halothane anesthesia. We used Farooque's technique to achieve a reproducible incomplete injury to the spinal cord (4). The dorsal surface of the cord was compressed with a steady load of 20 g for 5 min at the T8 level. Hematopoietic stem cells in phosphate-buffered saline or buffer alone (control) were injected into the spinal cord 1 week after injury. All animals were treated and cared for in accordance with the Chiba University School of Medicine guidelines pertaining to the treatment of experimental animals. We evaluated their functional outcome using hind limb motor function score (4) every week for total 5 weeks after the transplantation. The scores were analyzed by means of a parametric analysis of variance with repeated measures. Fluorescent in situ hybridization (FISH): We identified male transplanted cells in the injured spinal cords of female recipients by the demonstration of the presence of the Y chromosome in cells. Hybridization with a direct rhodamine-labeled chromosome Y DNA probe was recommended by the manufacturer of the probe (Oncor).

Double immunofluorescence staining: To identify the cell types of β-galactosidase expressing transplanted cells, we performed immunofluorescence staining (5). Omitting primary or secondary antibodies controlled the specificity of staining procedures. The primary antibodies used were as follows: rabbit anti β-galactosidase, mouse anti nestin (neural precursor), mouse anti NeuN (neuron), mouse anti GFAP (astrocyte), or mouse anti CC-1 (oligodendrocyte).

Results

Significant recovery of hind limb motor function score was detected in mice transplanted with hematopoietic stem cells compared with control. FISH findings showed that the transplanted cells containing Y chromosome positive nuclei survived and were distributed throughout the injured spinal cords (Fig. 1). Double staining showed that some β-galactosidase-positive cells simultaneously expressed nestin, GFAP and CC-1, suggesting that the transplanted cells differentiated into neural precursors, oligodendrocytes and astrocytes. We did not detect β-galactosidase-positive cells expressing NeuN, a specific marker for neuron.

Discussion

The present results demonstrate that transplantation of hematopoietic stem cells into spinal cord 1 week after contusion injury significantly improves functional outcome as measured on hind limb motor function score. Histological analysis revealed that the transplanted cells survive and differentiate into cells expressing neural markers 5 weeks after the transplantation. Hematopoietic stem cell fraction of bone marrow offers several advantages for generalizing cell transplantation methods. Clinical applications of fetal embryonic and neural stem cells are limited from both immunological and ethical standpoints. In contrast, transplantation of patient’s own bone marrow cells could circumvent the problems of host immunity and graft-versus-host disease. Our data suggest that transplantation of hematopoietic stem cells from bone marrow may represent an effective strategy for the treatment of spinal cord injury.