Intrathecal injection of autologous macrophages genetically modified to secrete BDNF by ex vivo electroporation improves hind limbs motor function after thoracic spinal cord injury in rats.

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Introduction. Spinal cord injury is one of the most serious conditions in the field of orthopedic surgery. The application of neurotrophic factors, such as GDNF and BDNF, are possible therapeutic methods. In the injured spinal cord, however, blood flow in the nervous tissue decreases remarkably (1). When neuroprotective substances are added intravenously, only a small amount of the substances reach the injured portion of the spinal cord. In addition, the half lives of most proteins in vivo are relatively short. Therefore, systemic intravenous administration of neurotrophic protein may not be an efficient way to treat damaged spinal cord tissue.

Direct infusion of neurotrophic protein into the neural parenchyma using pumps is another approach to treatment. However, several limitations should be considered as follows: 1) The spread of the proteins throughout the neural parenchyma is often limited. 2) The chronic implantation of the canula in the parenchyma results in the formation of a new scar at the insertion site. 3) The implanted canula may induce inflammation or clogging of the infusion device. 4) Continuous outflow of liquid can cause additional damage at the insertion site.

To develop a novel system for substance delivery to damaged ischemic tissue, we focused on the tissue-migration ability of macrophages. Macrophages migrate into damaged tissue or inflammatory tissue. For this study, gene transfer by ex vivo electroporation, a non-viral gene transfer method, was performed on autologous macrophages, and the cells were injected into the subarachnoid space.

Materials and methods. In this study, we used pEGFPLuc Vector (Clontech Inc. USA). This vector was constructed by inserting the GFP gene under over-expression of GFP protein (for vehicle animal). The cDNA for human BDNF was inserted in this vector for over-expression of BDNF protein (for BDNF animal). Intraperitoneal autologous macrophages were harvested from male Wistar rats (350 g-weight), and then vectors were transferred into the cells by electroporation using electroporator (CUY 21, NEPA GENE Co., Japan).

Rut spinal cord injury was performed at the 11th thoracic vertebral level using a MASCIS impacter. The gene-transfected autologous macrophages were injected into the subarachnoid space at the 4-5th lumbar intervertebral level just after the spinal cord injury. Hind-limb motor function was assessed with the Basso, Beattie and Bresnahan (BBB) scoring scale (2). The BBB scale is a 21-point scale that ranks no locomotion as 0 points and a normal gait as 21 points. For the histological examination, axial frozen sections with a thickness of 20 μm were produced. Then autofluorescence of GFP was assessed to identify the migrated macrophage in the spinal cord. Immunostaining with anti-BDNF antibody was performed to confirm successful over-expression of BDNF protein. Cy3-conjugated second antibody was used for color development for BDNF staining.

Results. Injected macrophages migrated and concentrated in the injured portion of the spinal cord and express GFP and BDNF proteins (Fig. 1). Most of the GFP/BDNF-positive cells were detected in the gray matter, especially in the area peripheral to the cavity caused by necrotic cell death. Fluorescence intensity increased with time and peaked at three weeks after the injury/injection. Autofluorescence was detected even 2 months after the injury/injection. The hind-limb function, evaluated by BBB scale, in the BDNF-gene transferred animals was significantly better than that in the vehicle animals from two weeks to eight weeks after the SCI (Fig. 2).

Discussion. Direct implantation of several kinds of cells into the damaged spinal cord has been reported. These reports show that implantation of several kinds of cells into the damaged nervous tissue may promote neuronal regeneration. The candidates for donor cells for spinal cord injuries are bone marrow stromal cells (3), macrophages (4) and neural stem cells (5). However, direct injection of the cells into the spinal cord may be a dangerous procedure for clinical use. Recently, more less-invasive transplantation procedures have been reported. Lepore et al. (6) reported that neural stem cells migrated into the damaged spinal cord tissue via intrathecal injection. They also tested the intravenous injection of neural stem cells. Some stem cells reached the damaged spinal cord tissue, although not as many as in the intrathecal injection procedure. These results suggest that the cells, which have migration activity similar to immature stem cells, are led to the damaged tissue. Macrophages, immune cells which are distributed widely in the body, have strong migratory abilities. In the vessels, they usually exist as monocytes. There are about 4 x 10^5 cells per liter of blood. When inflammation occurs, they migrate into the damaged tissue and change into macrophages. Macrophages are available in injured nervous tissue after spinal cord or peripheral nerve injuries (7). Inflammation after an injury may be an inducer of monocyte/macrophage migration. The hypothesis of this study was that if autologous macrophages exist in the subarachnoid space after a spinal cord injury, they may migrate and concentrate in the center of the inflamed or damaged area. This hypothesis has proven to be correct. This method is much safer than direct injection of the cells into the injured area.

The advantages of our novel substance delivery system are as follows: 1) Risk of virus activity can be avoided using ex vivo electroporation method. 2) Focused substance delivery to ischemic injured site can be achieved by macrophage migration. 3) Substance delivery may finish by the end of natural duration of macrophage life. 4) Since the procedure is simple and not so invasive, repetitive transplantation is clinically possible. In summary, we successfully transfected the BDNF gene into the damaged spinal cord via gene-transfected autologous macrophages by intrathecal injection. And the BDNF gene-transferred rats improved hind limb motor function after spinal cord injury. This method may be a useful substance-delivery system for the treatment of spinal cord injury.