**AC133 POSITIVE CELLS DERIVED FROM HUMAN PERIPHERAL BLOOD AND UMBILICAL CORD BLOOD PROMOTE CORTICOSPINAL AXON GROWTH IN ORGANO-TYPIC CO-CULTURES**

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**INTRODUCTION**

AC133 and CD34 are markers for human hematopoietic stem and progenitor cells. AC133 positive cells are considered to be more immature than CD34 positive cells. In addition, AC133 positive cells are able to differentiate into neural cells, and secrete several humoral factors which have beneficial effects for the axonal regeneration. To investigate the effects of AC133 positive cells on corticospinal axon growth, AC133 positive cells derived from human peripheral blood (PB) and umbilical cord blood (UCB) were transplanted to our original organotypic co-cultures of brain cortex and spinal cord. The purpose of this study was to assess the effect of AC133 positive cells on corticospinal axon growth quantitatively, and to clarify the mechanism of axon regeneration using the organotypic co-culture system.

**MATERIALS AND METHODS**

Isolation of AC133 Positive Cells from Peripheral blood and cord blood

PB cells were collected from healthy human donors. UCB cells were obtained with consent of the mothers had the caesarian operation. Mononuclear cells (MNCs) were separated from peripheral blood and cord blood by the gradient centrifugation. AC133 positive cells were isolated from the MNCs obtained from PB and UCB, using magnetic beads coated with anti-AC133 antibody according to manufacturer’s instructions (MACS).

Organotypic Co-Cultures

Organotypic co-cultures were prepared from the brains and the spinal cords of SD rats aged postnatal day 3. The brains were sectioned using a Vibratome. The region of the sensorimotor cortex was dissected from the coronal sections, and the thoracic spinal cord was bisected in the sagittal plane using a razor blade. The dissected cortex and spinal cord were placed on the membranes in the serum based medium. On the following day, the spinal cord pieces were aligned next to the white matter of the cortex. The co-cultures were incubated for up to 14 days. Only PBS were added to the co-cultures just after the cortical tissue and the spinal cord tissue contacted one another (control group). MNCs derived from PB or AC133 positive cells derived from PB and UCB were transplanted into the co-cultures just after the cortical tissue and the spinal cord tissue contacted one another (MNC group, AC133-PB group, AC133-UCB group).

Tracing of axon growth

Axonal projections from the cortex to the spinal cord were labeled by anterograde tracing with DiI. To analyze axonal growth, we counted the number of labeled axons passing through a reference line running parallel to the junction between the cortex and the spinal cord at a distance of 500, 1000, 1500 and 2000µm from the junction. All fibers crossing the reference line were counted, and the counts were expressed as an average number of axons per culture. Results were expressed as mean ± standard error. The statistical significance of differences in parameters was assessed by Kruskal-Wallis test with Scheffe post-hoc test.

Immunohistochemistry

To detect the transplanted cells, the co-culture tissues in the AC133-PB group were stained for human nuclei. Additionally, to identify whether the AC133 positive cells differentiated into neural or glial cells, the co-culture tissues in the AC133-PB group were stained for nestin, MAP2, GalC and GFAP at 7 days after transplantation.

**RESULTS**

In the control group, the average number of labeled axons that extended 500µm and 1000µm past the junction was 11.1 ± 1.3 and 2.8 ± 0.6 (mean ± S.E.). In the MNC group, axonal growth was not significantly different from that in the control group (Fig.1, 2). In the AC133-PB group and the AC133-UCB, axonal growth was enhanced compared to that in the control group (Fig.1). The average number of labeled axons that extended 500µm and 1000µm past the junction was 40.3 ± 7.2 and 17.5 ± 4.4 in the AC133-PB group, 56.4 ± 4.4 and 30.2 ± 4.2 in the AC133-UCB group. The average number of labeled axons in the AC133-PB group extending 500µm, 1000µm or 1500µm from the junction was significantly greater than in the control group (Fig.2).

**DISCUSSION**

Neurons and oligodendrocytes derived from AC133 positive cells were not observed. Therefore, we speculated that humoral factors released from AC133 positive cells might participate in the axonal growth.

**CONCLUSION**

The present study demonstrates that the transplantation of AC133 positive cells derived from peripheral blood and umbilical cord blood promotes corticospinal axon growth in organotypic co-cultures.