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Quantity and Distribution of de novo Generated Cells in the Spinal Cord of Adult Macaque Monkeys

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The existence of multipotent progenitors in the adult forebrain of mammals, including primates, has been widely documented. However, the data on the spinal cord are limited. We used bromodeoxyuridine (BrdU) to label *de novo* generated cells in adult (5-10 year old) non-human primates. We studied the quantity and distribution of BrdU-positive cells on histological sections from cervical, thoracic and lumbar spinal cord segments at 2h, 2, 5 or 10 weeks after exposure to BrdU. We report a significant reduction of BrdU-labeled cells at the 2/5/10-week post-injection time points as compared to the 2-hour time point. Further, we performed a subregional tracing of the BrdU-stained cells in gray matter, white matter, and around the central canal. This represents the first analysis of *in vivo* cell proliferation in normal adult primate spinal cord.

Key words: BrdU, proliferation, spinal cord, adult.

Introduction

Recently, mounting evidence using both i*n vitro* and *in vivo* experiments suggested the existence of adult stem cells in the mammalian brain. These cells are self-propagating and capable of producing all major cell types in the nervous system. In the spinal cord, adult stem/progenitor cells can be isolated, expanded and differentiated *in vitro* [7, 8]. Evidence suggests that a stem-like cell type can be localized in or near the ependymal layer of the spinal cord [1, 3]. Ependymal astrocytes appear the primary source of stem cell activity *in vitro* [2]. While most studies use rodent models, evidence in primates regarding the existence of spinal cord progenitors is scarce.

The aim of this study was to investigate the quantity and distribution of *de novo* generated cells at different levels and zones in the intact spinal cord of adult non-human primates (macaque monkeys).

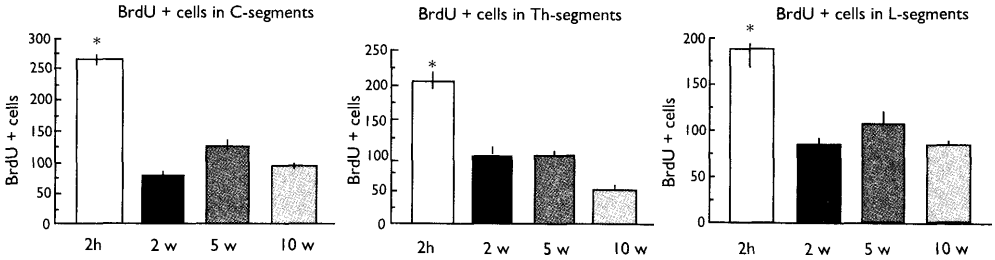

Fig. 1. Total number of BrdU+ cells on histological sections from cervical (C), thoracic (Th) and lumbar (L) segments of spinal cord 2 hours, 2, 5 and 10 weeks after BrdU injection.* P<0.05, Tukey-Kramer test.

Materials and methods

Female monkeys (*Macaca fuscata*) were used in accordance with the institutional guidelines of the Kanazawa University (Japan). Eleven adult (5-9 years-old) monkeys received daily injections (100 mg/kg, i.v.) of BrdU for 5 consecutive days. The animals in the short-term survival group (n = 3) were sacrificed 2 h after the last injection. The animals in the long-term survival groups were sacrificed 2 weeks (n = 3), 5 weeks (n = 3) and 10 weeks (n = 2) after the last injection. Under general anesthesia, the monkeys were intracardially perfused with 4% paraformaldehyde in 0,1 M phosphate buffer saline, pH 7,4. Spinal cord tissues were postfixed for 2 – 3 h in paraformaldehyde and cryoprotected. After embedding, transverse 40 μm thick sections were sequentially cut and stored at -20°C until staining. For BrdU labeling, DNA was denaturated in 50% formamide/2x SSC buffer and 2N HCl for 30 minutes at 37°C and mouse anti-BrdU (1:100) or rat anti-BrdU (1:100) first antibodies was then applied followed by appropriate secondary antibodies conjugated to biotin.

Image analysis of BrdU+ cells was performed on every 12th section from cervical, thoracic and lumbar segments of all groups with an Axovert S100 microscope (Carl Zeiss Co, Tokyo). We counted all BrdU+ cells on the section. Further, the gray matter and white matter were divided into specific regions of interest as follows: 1) gray matter (ventral horn, dorsal horn, central canal); 2) white matter (anterior funicle, lateral funicle, posterior funicle). Statistical analysis was performed using one-way ANOVA followed by Tukey – Kramer's post hoc comparisons and two-sided *t* test. Data expressed as the mean ± SEM. Differences were considered significant when $p \leq 0.05$.

Results

The total number of BrdU+ cells per section showed a significant reduction in the monkey groups with a long-term (weeks) survival after BrdU in all investigated spinal cord segments with more cells in the gray matter compared to the white matter (Fig. 1). Within the three zones of the gray matter we found more positive cells in the ventral horns of the cervical segments in short-term survival group and in lumbar segments 2 h and 10 weeks post infusion (Fig. 2). In the dorsal horns of gray matter, the number of BrdU+ cells was significantly higher in the 2h-survival group, in all segments. In the zone around the central canal, we found more BrdU+ cells only in the cervical segments of short-term (2h) survival group. There was no significant difference in the number of cells from the thoracic segments between experimental groups.

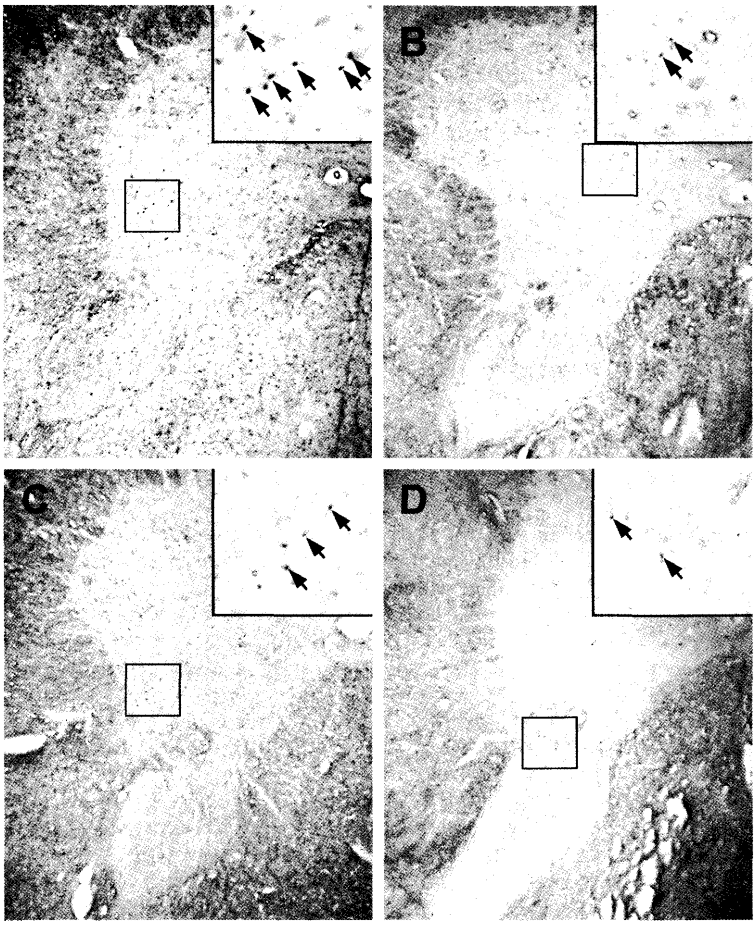

Fig. 2. Micrographs of BrdU+ cells in the spinal cord at various time points after BrdU injection: **A** – 2 hours, **B** – 2 weeks, **C** – 5 weeks, **D** – 10 weeks. Arrows indicate BrdU+ cells in the boxed areas, x20.

In the anterior column, more BrdU+ cells were present at 2h after BrdU in all segments. In the lateral column, the number of *de novo* generated cells was increased only in the cervical and thoracic segments of the short-term (2h) survival group. There was no difference in the number of cells in the dorsal column of all four experimental groups at all investigated levels.

In the other experimental groups, there was no significant difference in the total number of positive cells between the gray and white matter at all levels of the spinal cord with a few exceptions. In the third experimental group (5 weeks after injection), an increased number of *de novo* generated cells was found in the ventral horns of gray matter and anterior column of white matter in cervical segments. In the fourth experimental group (10 weeks after injection), the total number of BrdU+ cells in cervical and lumbar segments was increased both in the gray and white matter. In the gray matter, more cells were found in its ventral and dorsal horns. In the white matter, proliferative cells were concentrated in its anterior and lateral columns.

Discussion. We present the first analysis of *in vivo* cell proliferation in normal adult primate spinal cord. We found more *de novo* generated cells at 2h after BrdU than at 2/5/10 weeks. More BrdU+ cells were registered in cervical than in thoracic or lumbar segments. Increased number of *de novo* generated cells was found only in the gray matter of cervical segments and it was at the expense of more proliferating cells in the ventral horns. The presence and distribution of BrdU immuno-positive cells is indicative for an existing proliferation occurring at a higher rate in the gray matter and mainly at cervical level of the spinal cord. Our findings are consistent with data from other mammals [5, 7, 8]. The higher number of positive cells 2h after BrdU application compared to other groups corresponds to the division rate of newly generated cells that incorporate BrdU. We consider that the possible origin of stem cells in the spinal cord might be the ependymal zone as suggested by others [2, 4, 6]. It remains to be determined what is the cellular lineage of the proliferating cells, their pathways of migration and differentiation.

References

1. Chiasson, B. J., V. Tropepe, C. M. Morshead, D. van der Kooy, 1999. Adult mammalian forebrain ependymal and subependymal cells demonstrate proliferative potential, but only subependymal cells have neural stem cell characteristics. – J. Neurosci., 19, 4462– 4471.
2. Cizkova, D., M. Nagyova, L. Slovinska, I. Novotna, J. Radonak, M. Cizek, E. Mechirova, Z. Tomori, J. Hlucilova, J. Motlik, I. Jr. Sulla, I. Vanicky, 2009. Response of ependymal progenitors to spinal cord injury or enhanced physical activity in adult rat. – Cell Mol. Neurobiol., 29, 999–1013.
3. Doetsch, F., I. Caille, D. A. Lim, J. M. Garcia-Verdugo, A. Alvarez-Buylla, 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. – Cell, 97, 703–716.
4. Hamilton, L. K., M. K. Truong, M. R. Bednarczyk, A. Aumont, K. J. Fernandes, 2009. Cellular organization of the central canal ependymal zone, a niche of latent neural stem cells in the adult mammalian spinal cord. – Neuroscience, 164(3), 1044-1056.
5. Horner, P. J., A. Power, G. Kempermann, H. G. Kuhn, T. D. Plamer, J. Winkler, L. Thal, F. Gage, 2000. Proliferation and differentiation of progenitor cells through the intact adult rat spinal cord. – J. Neurosci., 20(6), 2218–2228.
6. Meletis, K., F. Barnabé-Heider, M. Carlén, E. Evergren, N. Tomilin, O. Shupliakov, J. Frisén, 2008. Spinal cord injury reveals multilineage differentiation of ependymal cells. – PLoS Biol. 6(7), e182.
7. Shihabuddin, L. S., J. Ray, F. H. Gage, 1997. FGF-2 is sufficient to isolate progenitors found in the adult mammalian spinal cord. – Exp. Neurol., 148, 577–586.
8. Weiss, S., C. Dunne, J. Hewson, 1996. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. – J. Neurosci., 16, 7599–7609.