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Bone marrow: a possible alternative source of cells in the adult nervous system

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Abstract

There is increasing evidence that stem cell populations can undergo a transition between mesodermal and neural ectodermal cell fates. Bone marrow-derived cells have been shown to be extremely versatile: they can become brain and liver cells and muscle, while other mesodermal derived cells have been shown to migrate into the brain and differentiate into neurons. Moreover, under the appropriate conditions, neural stem cells can differentiate into hematopoietic and muscle cell fates. It is now well established that newly differentiated cell types are continuously generated from immature stem cells throughout development and in adult mammals, including humans. This review addresses the contribution that bone marrow-derived stem cells may play during neurogenesis. We transplanted male bone marrow into female recipients to track and characterize the Y chromosome containing cells in the CNS (central nervous system) of mice.

Keywords: Stem cell; Differentiation; Hematopoietic; Central nervous system

1. Introduction

The term “stem cell” describes cells that have some capacity for self-renewal and can give rise to cells other than themselves through asymmetric cell division. Neural stem cells, the self-renewing precursors of neurons and glia, are found in both the developing and adult nervous system and have remarkable plasticity. When grafted into the developing brain, fetal-derived stem cells migrate along with the host cells and differentiate into cell types specific for the target region (Brustle et al., 1997, 1998; Olsson et al., 1998; Wichterle et al., 1999). When stem cells obtained from the adult brain are reintroduced into the mature nervous system, these cells generate new neurons and glia that are appropriate to the region where they migrated. For example, when stem cells from the adult hippocampus are transplanted back into the hippocampus, these cells generate new neurons and glia that exhibit similar characteristics to cells typically found in the dentate gyrus (Gage et al., 1995; Suhonen et al., 1996). However, when transplanted into regions that are not typically thought of as neurogenic, such as the adult cerebellum or striatum, these same cells differentiate into glial cells and not into neurons (Gage et al., 1995; Suhonen et al., 1996). Because of this plasticity, neural stem cells have become the focus of intensive research aimed at developing transplantation strategies to promote neural recovery in the diseased or injured nervous system (Bjorklund and Svendsen, 1999; McKay, 1997).

Recently, Bjornson et al. (1999) demonstrated that genetically marked neural stem cells can adopt very different fates than their well-characterized neural derivatives. When transplanted into irradiated host mouse, these nervous system-derived cells differentiate into a variety of hematopoietic cells, including the myeloid and the lymphoid cell lineages, as well as more immature blood cells. Stem cells from different peripheral tissues show remarkable plasticity as well. Highly purified hematopoietic stem cells transplanted intravenously into lethally irradiated recipients have been shown to differentiate into muscle cells (Gussoni et al., 1999) and bone marrow-derived cells can differentiate into endothelial cells (Asahara et al., 1997; Shi et al., 1998).

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It is widely accepted that circulating T-cells, B-cells, and macrophages enter and migrate throughout the CNS (Hickey, 1999; Hickey et al., 1991; Knopf et al., 1998; Williams and Hickey, 1995). Moreover, there is increasing evidence that bone marrow progenitor cells also migrate into the brain. Previously, microglia were reported to have a hematopoietic origin (Theele and Streit, 1993) and Eglitis and Mezey (1997) demonstrated that when injected into the tail vein of previously irradiated recipient mice, donor bone marrow cells (a mixture of stromal and mesenchymal cells) enter the brain and differentiate into microglia and astrocytes. Recent studies reconfirm these findings and show that cultured mesenchymal stem cells of the bone marrow stroma can migrate throughout the brain and differentiate into astrocytes after being transplanted into the lateral ventricle or striatum of recipient mice (Azizi et al., 1998; Kopen et al., 1999). Current efforts by several independent laboratories include determining whether other bone marrow-derived stem cells (e.g., hematopoietic stem cells) are similarly capable of differentiating into distinct neural cell types. Other mesodermal-derived cells are also capable of differentiating into cell types that were previously thought to be exclusively ectodermal in origin. For example, luteinizing hormone-releasing hormone neurons in mammals are unique among hypothalamic neurons in that they originate outside of the CNS and are of mesodermal origin (Schwanzel-Fukuda, 1999).

2. Results

Several of our recent studies suggest that at least some of the neurons and glia present in the adult nervous system may be bone marrow derived (Mezey et al., unpublished data). We used mice homozygous for a mutation in the *PU.1* gene as bone marrow recipients. PU.1 is a member of the family of transcription factors and is expressed exclusively in cells of the hematopoietic lineage. In the absence of donor bone marrow cells, mice homozygous for a disruption in the PU.1 binding domain lack macrophages, neutrophils, mast cells, osteoclasts, B and T cells at birth (McKercher et al., 1996; Tondravi et al., 1997). PU.1 null animals are born alive but die of severe septicemia within 48 h after birth. However, when these mice receive intraperitoneal injections of bone marrow at birth, containing both hematopoietic and stromal cell populations, they develop normally, are fertile, and live into adulthood. All of bone marrow cells are derived from the donor (without having to irradiate the recipients prior to transplantation), making these mice a useful model for optimizing the detection of donor cells that migrate into the nervous system from the general circulation.

Male donor cells were identified in the nervous system of female recipients by the demonstration of the presence of the Y chromosome in cells. A combination of non-radioactive in situ hybridization (to detect the Y chromosome) and immunohistochemistry (to visualize individual cell types) was used. Glial fibrillary acidic protein (GFAP) was used to identify mature astrocytes (Eng et al., 1971) and immunoreactive stem cells that line the lateral ventricle (Wichterle et al., 1999). NeuN, a nuclear marker that is localized exclusively to neuronal nuclei (Mullen et al., 1992; Sarnat et al., 1998; Wolf et al., 1996), was used to examine the co-localization of the Y-chromosome in neurons.

Marrow-derived cells (i.e., Y chromosome positive) were present in the CNS of all of the transplanted mice examined. The Y chromosome bearing cells were evenly distributed throughout the different brain regions, in both white and gray matter. Up to 5% of all cells (e.g., all identifiable nuclei) were Y chromosome positive. Interestingly, a large number of Y chromosome positive nuclei were present in cells within the choroid plexus of the lateral ventricle (Figs. 1C and 2C), in the ependyma of the ventricular system (Figs. 1C–D and 2C–D), and in the subarachnoid space. Many of these Y positive cells were associated with GFAP immunoreactivity (Fig. 2C–F), suggesting that at least some of the GFAP positive stem cells that are found in neurogenic regions originate in the bone marrow. An abundance of astrocytes that also labeled with the Y chromosome (Fig. 2E–F) were found throughout the CNS, suggesting that bone marrow-derived cells can migrate and differentiate appropriately. Our preliminary data suggest that one to two neurons for every 100 were also Y-positive when using the neuronal nuclear marker, NeuN. We were surprised to find that the ratio of all cells (including glial cells) to neurons is much lower than the commonly cited ratio of 10 (glial cells):1 (neurons). This number also appears in textbooks and is based on a reference from Nature (Correspondent, 1974). However, when one looks for quantitative histological data in the literature, our numbers are consistent with published thorough quantitative analyses of rat and mouse brain regions (Borges and Berry, 1978; Gabbott and Stewart, 1987; Heumann et al., 1977; Ling and Leblond, 1973; Ren et al., 1992), showing that the ratio of glia cells to neurons is in fact between 0.4 and 1.64. In our studies Y positive cells were also present within white matter tracts, including the corpus callosum, and their nuclei (as seen in DAPI (4′,6-diamidino-2-phenylindole dihydrochloride) staining) assumed the characteristic beaded pattern of oligodendrocytes. Due to the lack of well characterized nuclear or strictly cell body markers for oligodendrocytes (that would not label myelin) the actual localization of the Y chromosome in oligodendrocytes is difficult. Our current efforts are focused on definitively identifying bone marrow-derived oligodendrocytes using “tagged” donor tissue from different genetic mouse models.

We did not observe an increased density of Y chromosome positive cell nuclei in neurogenic regions, including the subventricular zone, olfactory migratory region, or hippocampus. However, in contrast to neural stem cells,

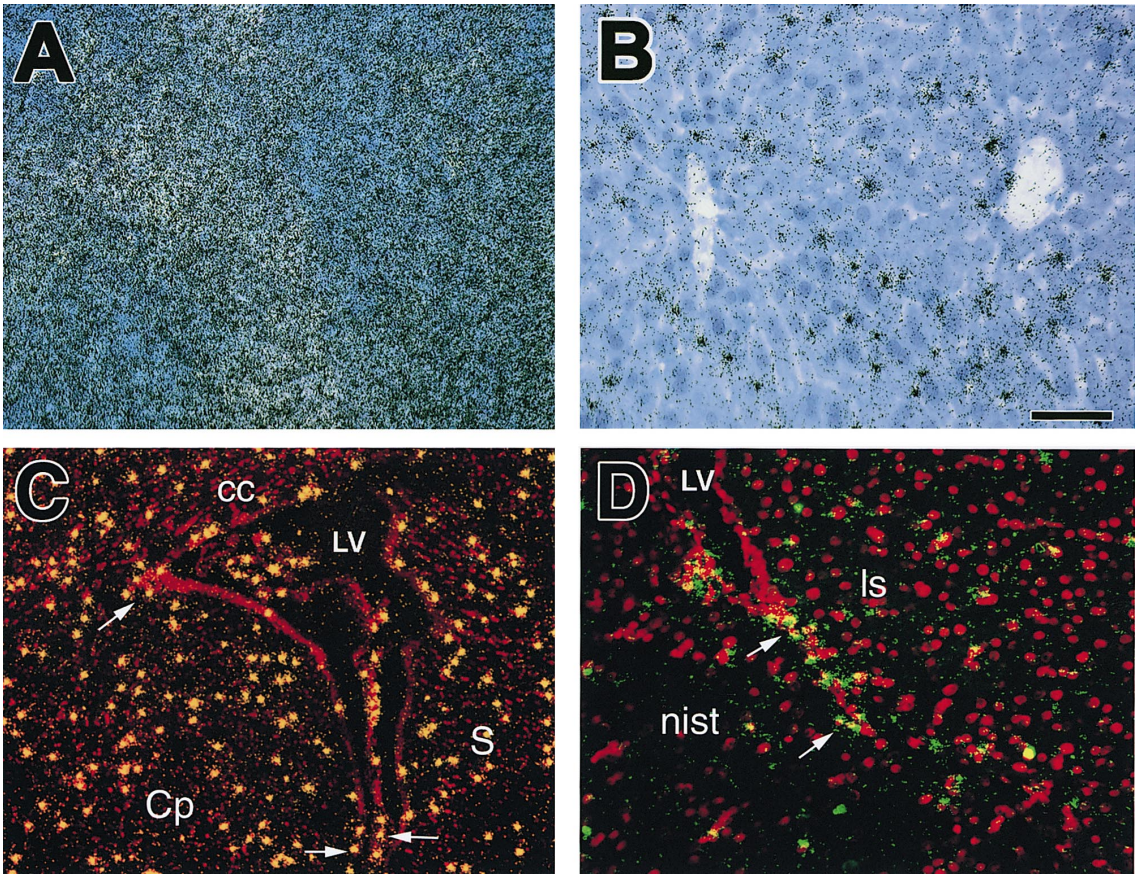

Fig. 1. (A) and (B) are sections of a spleen and a liver, respectively, of a recipient female PU1 knockout (PUKO) mouse that received the male bone marrow transplant at birth and was killed at two months of age. The Y chromosome probe was labeled using 35S-UTP and the section was hybridized with this radioactive probe, covered with nuclear emulsion and developed 3 days later. Note that the majority of the spleen cells and many liver cells are Y positive suggesting a high level of engraftment. The hematopoietic organs of all transplanted mice looked similar. (C) and (D) show radioactive in situ hybridization of the Y chromosome in coronal sections at the level of the septum. The nuclei are stained red with ethidium bromide and the grains over the Y chromosome positive cells are detected using darkfield illumination. Note the high number and rather homogeneous distribution of the labelled cells in both grey and white matter. Also note the very high density of positive cells in the choroid plexus of the lateral ventricle (C), and in the ependyma and subependymal zone (C and D) indicated by arrows. Scale bar: 50 μm (A,B), 200 μm (C) and 100 μm (D). Abbreviations: cc = corpus callosum, Cp = caudate-putamen, ls = lateral septal nucleus, LV = lateral ventricle, nist = bed nucleus of the stria terminalis.

mesodermal stem cells differentiate into microglia (Theele and Streit, 1993). Therefore, since all microglia in the recipient animals are also Y chromosome positive, any regional differences in the number of Y positive neurons would be masked by the significantly higher number of homogeneously distributed microglia.

Together with previously published studies, our finding suggest that in addition to microglia, some neural cells may also be derived from the mesoderm. There is increasing evidence that bone marrow cells migrate throughout the brain and differentiate into neurons and glial cells, thereby suggesting that the blood can supply the brain with an alternative source of neural cells. Up until recently, neurons and macroglia (oligodendrocytes and astrocytes) were thought to arise exclusively from pluripotent neural stem cells that are present both in the developing (Mujtaba et al., 1998) and adult CNS (Doetsch et al., 1999; Gage, 2000; Johansson et al., 1999; Weiss et al., 1996b; Weiss and van der Kooy, 1998). In adult mammals, for every two thousand existing neurons one new neuron is produced each day (Gage, 2000; Kempermann et al., 1997). Where do these new neurons come from? In the rodent brain at least two well-characterized germinal centers, characterized by high-density cell division, are thought to be responsible for replenishing lost or dying neurons throughout ontogeny. The subgranular zone of the dentate gyrus gives rise to hippocampal interneurons and the forebrain subventricular zone gives rise to cells that migrate into the olfactory bulb and differentiate into small interneurons (Morshead et al., 1994; Reynolds and Weiss, 1992; Weiss et al., 1996a). Recently, Johansson et al., (1999) provided evidence that neural stem cells in the adult ependymal cell layer lining the ventricles differentiate into glial cells and neurons. Moreover, in another study, glial fibrillary acidic protein immunoreactive cells present in the subventricular zone acted as neural stem cells in both the normal and regenerating brains (Doetsch et al., 1999). Bjornson et al., (1999) were able to turn brain into blood' when they

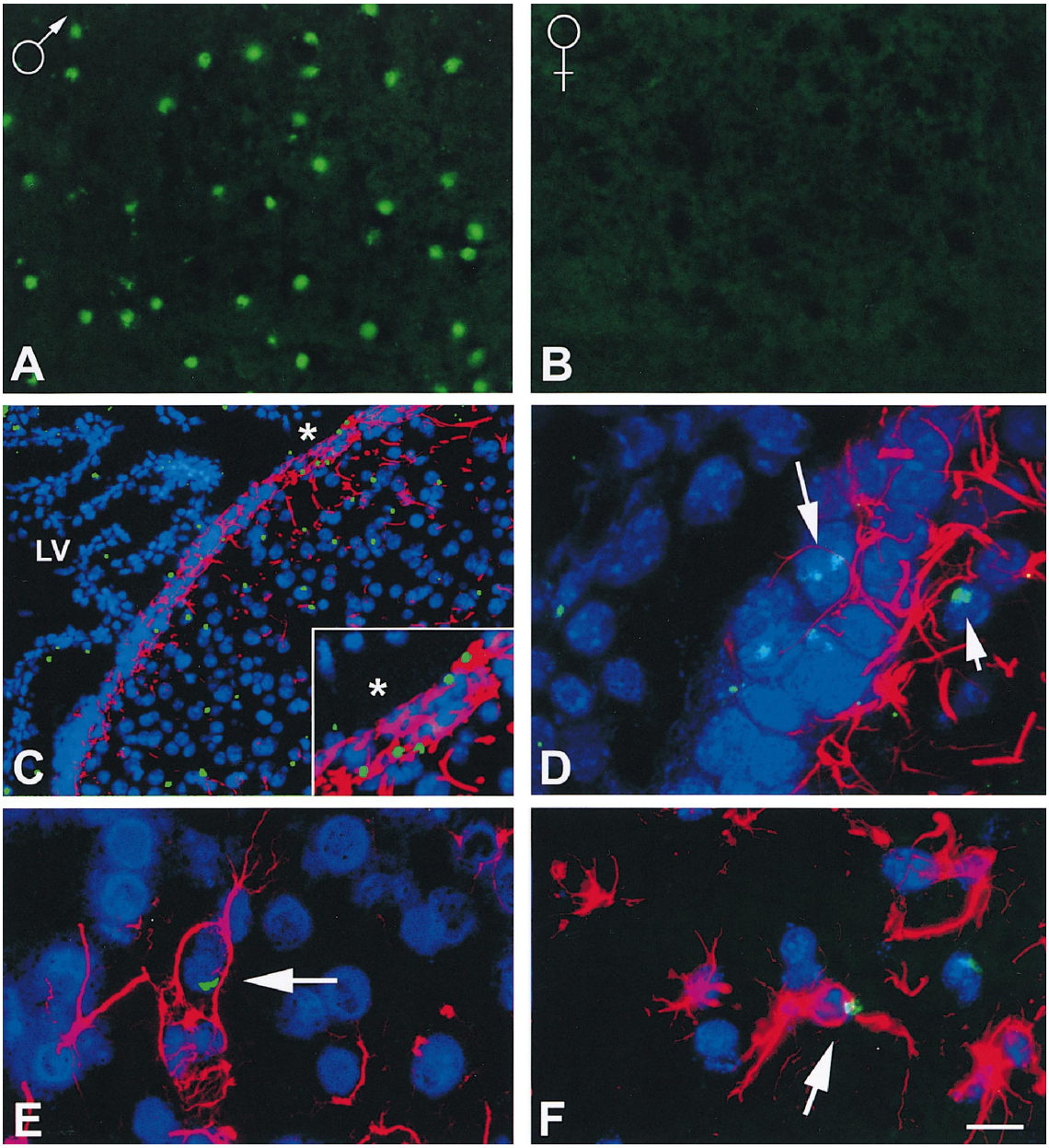

Fig. 2. (A) and (B) are images of a control male and control female brain section. These sections were mounted on the same slide and hybridized together to show the presence (A) and the lack (B) of the Y chromosome. In these sections the Y chromosome was detected using a digoxigenin labelled riboprobe, an anti-digoxigenin-peroxidase enzyme and the Tyramide–FITC conjugate. Note the location of the Y chromosome at the periphery of the nucleus due to its heterochromatic nature. (C) The coronal 10-μm-thick section shows the lateral ventricle and caudate/putamen of a female brain 3 months after transplant at the coronal level 200 μm behind the bregma using conventional fluorescent microscopy. Note the high density of Y chromosome positive cell nuclei (green dots — pseudocolored from alkaline phosphatase — purple) in the ependyma and in the subependymal zone surrounded by red astrocytic (GFAP positive) processes. (D) shows a confocal image (1.5 μm thick) of the ependyma and the subependymal area of a different animal using a 63× objective and a 2× digital magnification. The image demonstrates many Y positive nuclei among the GFAP processes (arrows). The Y chromosome here is detected by FITC–Tyramide. E and F demonstrate two examples of Y chromosome labelled astrocytes (arrows) from the cerebral cortex of mice 2 months after transplant. Scale bar: 25 μm (A,B), 50 μm (C), 10 μm (D–F). Abbreviation: LV = lateral ventricle, asterisk labels the ependymal area that is magnified in the inset.

showed that subventricular zone stem cells can become hematopoietic in nature when cultured in a certain way. Based on our data, we suggest that this plasticity is most likely bidirectional. Our studies indicate that there is a continuous influx of bone marrow progenitor cells into the ependymal and subependymal zones throughout life, which may give rise to a variety of CNS cell types, including microglia, new neural stem cells, neurons, oligodendrocytes, and astrocytes. Based on the distribution of the Y chromosome positive cells, we suggest that the circulating bone marrow progenitor cells reach the CNS through the choroid plexus, travel through the cerebrospinal fluid to

cross the ependyma, and seed in the subependymal zone. The cerebrospinal fluid environment may in fact provide the necessary environmental cues that initiate the differentiation of cells down a neural pathway. It is also intriguing to speculate that this influx of cells from the periphery might be a contributing factor of the CNS presentation of diseases that primarily originate in and affect the hematopoietic system (leukemia, acquired immunodeficiency syndrome etc.).

Additional growth factor treatment in vitro may be necessary to enrich, instruct, or select for distinct CNS cell types. Once these factors are identified, bone marrow-derived cells may become a useful source of cells in the treatment of neurodegenerative disease. Since at least two different types of stem cells have been isolated from bone marrow (hematopoietic and stromal) determining the potential for each of these populations to become neurons and glia will be an important step towards optimizing regenerative therapies. Moreover, future studies are needed to examine whether human bone marrow cells have a similar "neural" potential. In support of our in vivo findings, recent in vitro studies also suggest that both human (Reyes and Verfaille, 1999) and rodent (Sanchez-Ramos et al., 2000) adult bone marrow cells have the ability to differentiate into cells that express neuronal markers. If these findings are proven relevant in humans in vivo, then we will need to know if there are temporal limits dictating when cells can cross the blood–brain barrier and, if so, what are the optimal entry routes for transplanting cells. Other important issues include determining whether bone marrow-derived stem cells need to pass through the general circulation or be exposed to the cerebral spinal fluid of the central nervous system before acquiring their neural potential.

The therapeutic implications of these findings are significant. Bone marrow is far more accessible than neural stem cells and has the added advantage of having inherent host compatibility. There is also no need to screen for viral and foreign antigens before using these cells in the host. Moreover, the use of human bone marrow stem cells from adult donors would circumvent many of the ethical considerations surrounding the use of human fetal tissue.

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References

Asahara, T. et al., 1997. Isolation of putative progenitor endothelial cells for angiogenesis. Science 275, 964–967.

Azizi, S.A. et al., 1998. Engraftment and migration of human bone marrow stromal cells implanted in the brains of albino rats-similarities to astrocyte grafts. Proc. Natl. Acad. Sci. U. S. A. 95, 3908–3913.

Bjorklund, A., Svendsen, C., 1999. Stem cells. Breaking the brain–blood barrier. Nature 397, 569–570, [news].

Bjornson, C.R. et al., 1999. Turning brain into blood: a hematopoietic fate adopted by adult neural stem cells in vivo. Science 283, 534–537, [see comments].

Borges, S., Berry, M., 1978. The effects of dark rearing on the development of the visual cortex of the rat. J. Comp. Neurol. 180, 277–300.

Brustle, O. et al., 1998. Chimeric brains generated by intraventricular transplantation of fetal human brain cells into embryonic rats. Nat. Biotechnol. 16, 1040–1044.

Brustle, O. et al., 1997. In vitro-generated neural precursors participate in mammalian brain development. Proc. Natl. Acad. Sci. U. S. A. 94, 14809–14814.

Correspondent, 1974. A new role for the glial cell? Nature 251, 100–101.

Doetsch, F. et al., 1999. Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 97, 703–716.

Eglitis, M.A., Mezey, E., 1997. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc. Natl. Acad. Sci. U. S. A. 94, 4080–4085.

Eng, L.F. et al., 1971. An acidic protein isolated from fibrous astrocytes. Brain Res. 28, 351–354.

Gabbott, P.L., Stewart, M.G., 1987. Distribution of neurons and glia in the visual cortex (area 17) of the adult albino rat: a quantitative description. Neuroscience 21, 833–845.

Gage, F.H., 2000. Mammalian neural stem cells. Science 287, 1433–1438.

Gage, F.H. et al., 1995. Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc. Natl. Acad. Sci. U. S. A. 92, 11879–11883.

Gussoni, E. et al., 1999. Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401, 390–394.

Heumann, D. et al., 1977. Postnatal development of the mouse cerebral neocortex: II. Quantitative cytoarchitectonics of visual and auditory areas. J. Hirnforsch. 18, 483–500.

Hickey, W.F., 1999. Leukocyte traffic in the central nervous system: the participants and their roles. Semin. Immunol. 11, 125–137.

Hickey, W.F. et al., 1991. T-lymphocyte entry into the central nervous system. J. Neurosci. Res. 28, 254–260.

Johansson, C.B. et al., 1999. Identification of a neural stem cell in the adult mammalian central nervous system. Cell 96, 25–34.

Kempermann, G. et al., 1997. Genetic influence on neurogenesis in the dentate gyrus of adult mice. Proc. Natl. Acad. Sci. U. S. A. 94, 10409–10414.

Knopf, P.M. et al., 1998. Antigen-dependent intrathecal antibody synthesis in the normal rat brain: tissue entry and local retention of antigen-specific B cells. J. Immunol. 161, 692–701.

Kopen, G.C. et al., 1999. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc. Natl. Acad. Sci. U. S. A. 96, 10711–10716.

Ling, E.A., Leblond, C.P., 1973. Investigation of glial cells in semithin sections: II. Variation with age in the numbers of the various glial cell types in rat cortex and corpus callosum. J. Comp. Neurol. 149, 73–81.

McKay, R., 1997. Stem cells in the central nervous system. Science 276, 66–71.

McKercher, S.R. et al., 1996. Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. EMBO J. 15, 5647–5658.

Morshead, C.M. et al., 1994. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron 13, 1071–1082.

Mujtaba, T. et al., 1998. A common neural progenitor for the CNS and PNS. Dev. Biol. 200, 1–15.

Mullen, R.J. et al., 1992. NeuN, a neuronal specific nuclear protein in vertebrates. Development 116, 201–211.

Olsson, M. et al., 1998. Incorporation of mouse neural progenitors transplanted into the rat embryonic forebrain is developmentally regulated and dependent on regional and adhesive properties. Eur. J. Neurosci. 10, 71–85.

Ren, J.Q. et al., 1992. Quantitative analysis of neurons and glial cells in the rat somatosensory cortex, with special reference to GABAergic neurons and parvalbumin-containing neurons. Exp. Brain Res. 92, 1–14.

Reyes, M.C.M., Verfaille, 1999. Turning marrow into brain: generation of glial and neuronal cells from adult bone marrow mesenchymal stem cells. American Society of Hemtology 41st Annual Meeting. Abstract No. 1676.

Reynolds, B.A., Weiss, S., 1992. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 255, 1707–1710, [see comments].

Sanchez-Ramos, J., et al., 2000. Adult bone marrow stromal cells differentiate into neural cells in vitro. Seventh Annual Conference of the American Society for Neural Transplantation and RepairClearwater, FL, pp. 29; 26–21.

Sarnat, H.B. et al., 1998. Neuronal nuclear antigen (NeuN): a marker of neuronal maturation in early human fetal nervous system. Brain Dev. 20, 88–94.

Schwanzel-Fukuda, M., 1999. Origin and migration of luteinizing hormone-releasing hormone neurons in mammals. Microsc. Res. Tech. 44, 2–10.

Shi, Q. et al., 1998. Evidence for circulating bone marrow-derived endothelial cells. Blood 92, 362–367.

Suhonen, J.O. et al., 1996. Differentiation of adult hippocampus-derived progenitors into olfactory neurons in vivo. Nature 383, 624–627.

Theele, D.P., Streit, W.J., 1993. A chronicle of microglial ontogeny. Glia 7, 5–8.

Tondravi, M.M. et al., 1997. Osteopetrosis in mice lacking haematopoietic transcription factor PU.1. Nature 386, 81–84.

Weiss, S. et al., 1996a. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J. Neurosci. 16, 7599–7609.

Weiss, S. et al., 1996b. Is there a neural stem cell in the mammalian forebrain? Trends Neurosci. 19, 387–393, [see comments].

Weiss, S., van der Kooy, D., 1998. CNS stem cells: where's the biology (a.k.a. beef)? J. Neurobiol. 36, 307–314.

Wichterle, H. et al., 1999. Young neurons from medial ganglionic eminence disperse in adult and embryonic brain. Nat. Neurosci. 2, 461–466.

Williams, K.C., Hickey, W.F., 1995. Traffic of hematogenous cells through the central nervous system. Curr. Top Microbiol. Immunol. 202, 221–245.

Wolf, H.K. et al., 1996. NeuN: a useful neuronal marker for diagnostic histopathology. J. Histochem. Cytochem. 44, 1167–1171.