

# The therapeutic plasticity of neural stem/precursor cells in multiple sclerosis

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## Abstract

Adult multipotent neural stem/precursor cells (NPCs) have the capacity to self-renew and generate functional differentiated cells (*e.g.* neurons, astrocytes or oligodendrocytes) within discrete tissue-specific germinal niches, such as the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus of the hippocampus. Due to their intrinsic plasticity NPCs can be considered an essential part of the cellular mechanism(s) by which the central nervous system (CNS) tries to repair itself after an injury and, as a consequence, they also represents an attractive therapeutic tool for the treatment of neurological disorders. Here we will discuss not only the role of NPC-based transplantation therapies in multiple sclerosis (MS) but also recent data suggesting that endogenous NPCs, while contributing to CNS repair in MS, may also become the target of the disease itself.

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## 1. Introduction

### 1.1. The endogenous stem cell compartment of the adult brain

The adult mammalian central nervous system (CNS) harbours low numbers of multipotent stem and precursor cells within certain specialised tissue compartments defined as ‘germinal niches’ [1]. These cells display cardinal features such as unlimited capacity for self-renewal, indefinite ability to proliferate in response to mitogens, and multipotency for the different neuroectodermal lineages of the CNS [2]. Self-renewal and differentiation of stem and precursor cells are non-cell autonomous processes that are regulated by the specialized microenvironment of the germinal niche, in which these cells reside. Within the niche, both environmental cues and intrinsic genetic programs are required to direct/regulate stem and precursor cell proliferation and differentiation [1].

The subventricular zone (SVZ) of the lateral ventricle wall and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus are the two major germinal regions of the adult mammalian brain (Fig. 1).

In the SVZ, cells with structural and molecular characteristics of astrocytes are identified as the true stem cells (SVZ astrocyte or type B cells) [3–5]. They are immunoreactive for the glial fibrillary acidic protein (GFAP) and lie in intimate contact with the other cells of the SVZ niche, the so called type C cells (or transit-amplifying neuronal progenitors) and type A cells (or lineage-committed migratory neuroblasts). The usual cell lineage differentiation pathway goes from the GFAP<sup>+</sup> type B cell, through an intermediate GFAP<sup>+</sup>/distal-less homeobox (Dlx) 2<sup>+</sup> type C cell, to GFAP<sup>-</sup>/Dlx2<sup>-</sup>/Doublecortin (DCX)<sup>+</sup>/neural cell adhesion molecule (NCAM)<sup>+</sup> migratory neuroblast [3]. Interestingly, a ribbon of SVZ astrocytes lining the lateral ventricles and proliferating *in vivo* and *in vitro* as multipotent progenitor cells has also been described in the adult human brain [6]. Unlike rodents and non-human primates, the adult human SVZ appears to be somehow devoid of extensive chain migration or large numbers of newly formed young neurons

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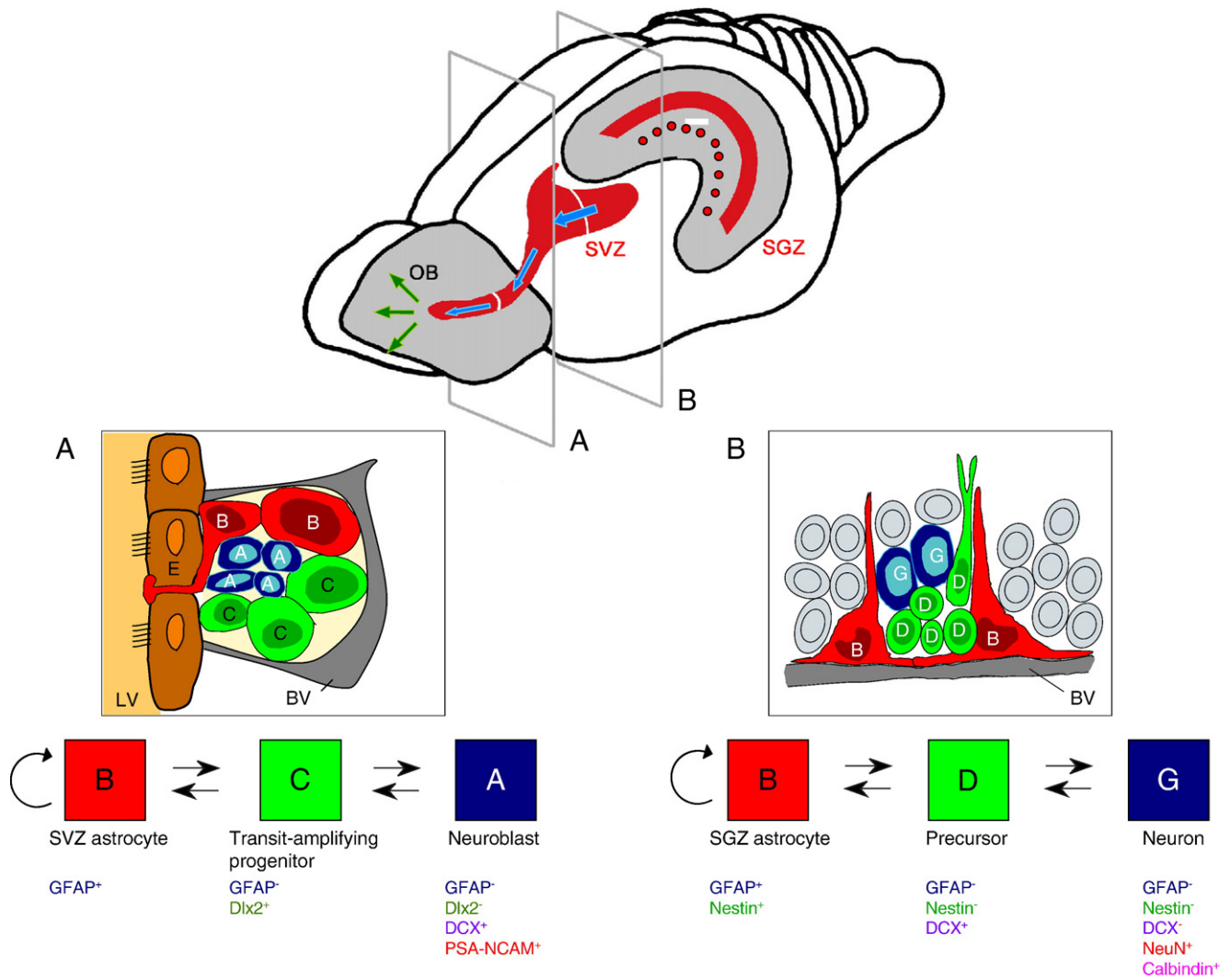


Fig. 1. Relationship between the three classes of adult neural stem and precursor cells (NPCs) in the two major germinal niches of the adult rodent brain, which are located in the subventricular zone (SVZ) of the lateral ventricles (A) and in the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus (B). Blue arrows indicate cell migration pathways, while green arrows indicate cell differentiation pathways. OB, olfactory bulb.

[6,7] and the SVZ human astrocytes are separated from the ependyma by an hypocellular gap [8].

In the SGZ, GFAP/Nestin-expressing astrocytes function as stem cells (type B cells), undergo self-renewal, proliferation and differentiation into transit amplifying DCX-expressing type D cells, which then differentiate further into lineage-committed migratory granule neurons (type G cells, immunoreactive for calbindin) [9,10]. The maintenance and differentiation of neural stem and precursor cells in brain germinal niches depends on their physical contact to the basal lamina that – acting as a scaffold – sequesters and/or modulates the release of discrete amounts of cytokines, growth factors and/or stem cell regulators, all released from local cells (e.g., fibroblasts, macrophages and pericytes) [11].

Additionally, also the central canal of the adult mammalian spinal cord has been shown to contain relatively

quiescent radial glia-like cells which may re- enter cell cycle and exhibit stem/progenitor cell properties upon injuries [12,13].

## 2. The brain repairs itself

In the early 20th century, the seminal work of Francisco Tello showed that the CNS has the ability to regenerate itself after an injury. This observation has been rejuvenated by detailed *in vitro* and *in vivo* mechanistic evidence supporting the existence of an innate self-maintenance program, ‘the brain repair system’, which sustains tissue homeostasis and repair [14]. This work led to the idea that further dissection of the molecular and cellular events that control the intrinsic brain repair mechanisms might provide an attractive framework for developing more efficacious therapies for neurological diseases.

Several molecular and cellular mechanisms involved in intrinsic brain repair have been described so far. They can be divided into three distinct, although strictly interrelated, categories. (i) Inflammation-driven processes – these contribute to brain repair if the humoral and cellular inflammatory components shift balance (function) over time from a tissue-damaging mode to a mode promoting tissue repair (*e.g.*, neurotrophic support from inflammatory cells); (ii) Brain plasticity – the recruitment of alternative “non-damaged” functioning neuronal pathways (cortical maps) mainly *via* axonal branching and synaptogenesis, occurs as a consequence of brain damage. Whether or not and to what extent recapitulation of developmental pathways is the underlying phenomenon sustaining brain plasticity is still matter of investigation; (iii) Endogenous adult neural stem/precursor cells – if these cells survive the inflammatory and/or degenerative insult, they may be capable of migrating within damaged areas and promoting repair *via* several mechanisms of action, such as cell replacement, remyelination, and/or neuroprotection [15]. In addition to the contribution of brain stem cells, there remains much debate as to whether there may also be a contribution to brain repair by stem cells of a different embryonic origin. A number of possible roles have been attributed to these cells, including cell replacement by trans-differentiation, fusion, and immunosuppression. However, continued study will be required to determine the relative contribution, if any, of these cells.

Brain repair spontaneously occurs in MS. This is supported by data from autopsy or biopsy studies indicating that almost 40% of MS lesions show evidence of remyelination [16]. Oligodendrocyte precursors (OPCs) play an unquestionable role in promoting remyelination [17,18] but there are data indicating that this is not the sole and exclusive cell-mediated mechanism underlying remyelination in MS. Owing to their ability to support neurogenesis and gliogenesis during adulthood [19], endogenous neural stem and precursor cells [hereafter referred to as adult neural precursor cells (NPCs)] might also contribute to remyelination in MS. In experimental models of chronic inflammatory multifocal demyelinating disorders such as

experimental autoimmune encephalomyelitis (EAE), the animal model of MS, mitotically active progenitor cells, have been shown to have their physiological destiny subverted. These cells normally reside within either the SVZ of the mouse and human brain or the subependymal layer of central canal of the rodent spinal cord, and follow the longitudinal migration along the rostral migratory stream (RMS) to the olfactory bulb (OB) or the radial migration to the lateral columns of the spinal cord, respectively [20–22]. Further, a four-fold increase of the number of type B cell-derived oligodendrocytes expressing the platelet-derived growth factor (PDGF) receptor  $\alpha$  and the oligodendrocyte lineage transcription factor 2 (Olig2) is observed as a consequence of the occurrence of a single demyelinating lesion within the corpus callosum [23]. Thus, accumulating evidence suggests that endogenous gliogenesis, driven by either OPCs and/or NPCs, may occur as part of an ‘intrinsic’ self-repair process during inflammatory demyelination. However, there remains no convincing explanations about the overall incapacity of the endogenous stem/precursor cell compartment in promoting full and long-lasting CNS repair in MS.

We have recently proposed that inflammation might derange the appropriate temporal and spatial relationship(s) between cells residing within the SVZ. This would lead to a prevalence of type B stem cells undergoing terminal symmetrical cell division, and might then explain the failure of remyelination at least in periventricular demyelinating MS lesions [15]. Experimental and human studies support such hypothesis. Inflammatory demyelinating lesions are frequently visible in MS through the lateral ventricular lining as elongated grey sleeves, are closed to subependymal veins, and are very often associated to a granular ependymitis. These lesions usually appear as wedged-shaped in coronal sections with a broad base toward the ventricles [24]. Moreover, although complete remyelination is present in some periventricular demyelinating MS lesions, the global extent of remyelination is lower in these lesions, when compared to lesions located in the deep white matter or subcortically [16]. MRI of human MS supports these

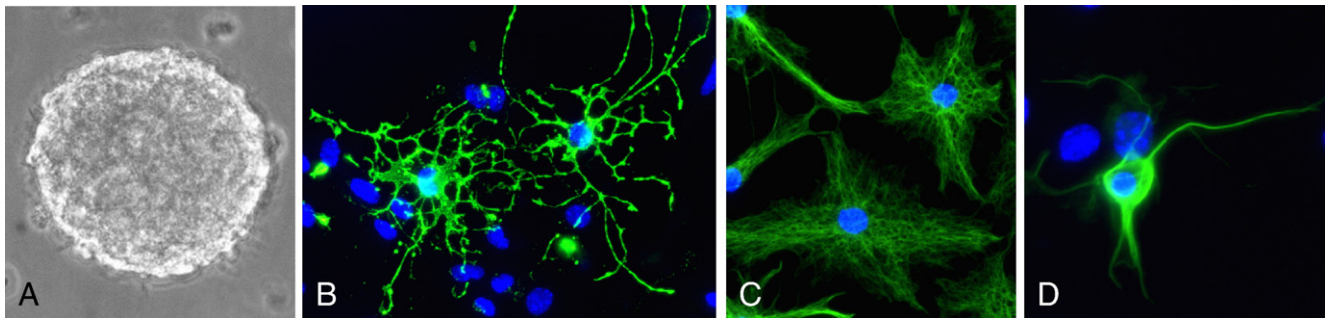


Fig. 2. *In vitro* characteristics of NPCs used for transplantation in CNS demyelinating disorders. Upon growth factor withdrawal, mouse NPCs – grown *in vitro* as free-floating neurospheres (A, phase contrast) – give rise to progeny containing variable percentages of oligodendrocytes [B, Galactocerebroside (GalC)], astrocytes [C, glial fibrillary acidic protein (GFAP)] or neurons (D, beta-tubulin-III). Nuclei in B–D have been counterstained with dapi (blue). Magnification 40 $\times$ .

histological findings. Gadolinium (Gd) enhancement in T<sub>1</sub>-weighted images, which are highly suggestive of site-specific inflammation, as well as hyperintense T<sub>2</sub>-weighted lesions, are frequently visible within the periventricular brain region. Moreover, spatial mapping of T<sub>2</sub> and Gd-enhancing T<sub>1</sub> lesion volumes in MS suggests that a proportion of the periventricular T<sub>2</sub> lesion volume may arise from mechanisms other than those associated with early breakdown of the blood-brain barrier leading to T<sub>1</sub> Gd enhancement. This discrepancy of T<sub>1</sub>/T<sub>2</sub> lesion distribution includes the possibility that the central white matter might have a greater susceptibility to persistent T<sub>2</sub> hyperintense changes following inflammation [25]. During sub-acute LPS-induced brain inflammation, interleukin (IL)-6 released by microglia significantly impairs neurogenesis in the hippocampus *in vivo*. However, the impairment is fully restored when non-steroidal anti-inflammatory drugs (such as indomethacin) are given [26]. *In vitro* generation of new neurons and oligodendrocyte from NPCs is induced and supported by mouse microglia that have encountered T cell-associated cytokines (such as interferon- $\gamma$  and IL-4), but blocked by those that have encountered endotoxins (such as LPS) [27].

Taken together, these data support the ensuing idea that inflammatory demyelinating disorders, such as MS, might be viewed as the consequence of the *dysfunction of the endogenous stem cell compartment* rather than caused by an uncontrolled, and still undiscovered, pathogenic alien(s). This hypothesis may also accounts for the heterogeneity of lesion development and composition as well as for the regional differences between lesions. If correct, the concept that NPCs residing within the SVZ are responsible for periventricular lesions, while multipotent OPCs dispersed throughout the entire CNS account for the lesions outside neurogenic areas, might not sound that provocative.

### 3. NPC transplantation in MS: the atypical ectopic niche

As a consequence the derangement of brain germinal niches in MS, one possible treatment approach would be to reconstitute the endogenous stem cell compartment by transplantation of functioning NPCs (Fig. 2). Importantly, reconstitution would need to take place in ectopic CNS areas owing to the fact that an *hostile inflamed microenvironment* is frequently found in major germinal niches in MS brains.

Some recent experiments have shown that in multifocal inflammatory CNS disorders (*i.e.*, stroke, MS, brain tumors, spinal cord injury, epilepsy, brain trauma, amyotrophic lateral sclerosis *etc.*) therapeutic somatic stem cells [*e.g.* bone marrow stem cells (BMSC), umbilical cord blood stem cells (UCBSC), mesenchymal stem cells (MSC), NPC] – injected through the blood stream or into the cerebrospinal fluid circulation – can specifically reach inflamed CNS areas where they persist for months and promote recovery [28–32]. The mechanism by which these cells, or at least those from neural origin, can specifically reach inflamed CNS areas has been recently elucidated in mice affected by

chronic or relapsing–remitting EAE. In these mice, intravenously (*i.v.*)- and intracerebroventricularly (*i.c.*)-injected NPCs specifically enter into inflamed areas of the CNS where they promote a marked reduction in demyelination, axonal loss and astrogliosis and – as a consequence – restore neurological functions [33–36]. Specific homing is due to the fact that NPCs constitutively express high-affinity cell adhesion molecules (CAM), such as CD44,  $\alpha$  and  $\beta$  integrins, as well as functional chemokine receptors (such as CCR1, CCR2, CCR5, CXCR3 and CXCR4) [34]. These molecules render NPCs capable of utilizing certain pathways used by immune cells to invade the inflamed CNS. CAM-expressing NPCs are, in fact, capable of responding to local cytokine release (*e.g.* IL-6, IL-1  $\beta$ , TNF- $\alpha$ ) in CNS areas of inflammation and, in turn, adhere spontaneously and migrate across to the inflamed endothelium. As a functional proof, *in vitro* pre-treatment of NPCs with neutralizing antibodies against  $\alpha 4$  integrin led to a reduction of around 50–60% of the number of injected NPCs accumulating within the brain and the spinal cord of EAE mice [34].

Once within inflamed CNS areas, systemically-injected NPCs accumulate (and persist) around the perivascular space where reactive astrocytes, inflamed endothelial cells and blood-borne infiltrating T cells co-reside. In these areas, dubbed ‘CNS atypical ectopic niches’, a molecular cross talk takes place between the different cells of the atypical niche.

On one hand, the great majority of transplanted NPCs survive long term, while displaying undifferentiated features (*e.g.*, round-shaped morphology and lack of major antigens of differentiation), owing to the focal release of stem cell regulators (*e.g.*, BMPs, Noggin) by immune cells and reactive astrocytes.

On the other hand, NPCs promote neuroprotection by *in situ* release of immunomodulatory molecules (*e.g.*, anti-inflammatory cytokines) and neurotrophic factors [*e.g.*, nerve growth factor (NGF), fibroblast growth factor (FGF)-II, ciliary neurotrophic factor (CNTF) and brain-derived neurotrophic factor (BDNF)] [15,33,34]. Via the release/expression of immunomodulatory molecule (*e.g.* FasL, Apo3L, TRAIL) NPCs promote apoptosis of effector cells expressing death receptors (*e.g.* encephalitogenic Th1 cells). Via the release of neurotrophic growth factor (*e.g.*, TGF  $\beta$  FGF-II), NPCs also contribute to significant reduction of glial scarring [15,33,34]. Last but not least, transplanted NPCs may also differentiate into myelin forming cells [33]. As a net effect of these different mechanisms of neuroprotection, five-fold increase in demyelinating areas of the number of ‘remyelinating’ endogenous OPCs is obtained [33].

### 4. Conclusions

Recent results consistently challenge the sole and limited view that neural stem/precursor cells therapeutically work exclusively throughout cell replacement. NPC transplantation may also promote CNS repair *via*

bystander mechanisms, mainly exerted by undifferentiated ‘stem’ cells releasing – at the site of tissue damage in response to environmental needs – a milieu of neuroprotective and immunomodulatory molecules. Thus, we can propose the concept of ‘therapeutic plasticity’, which can be viewed as the capacity of somatic stem cells to adapt their fate and function(s) to specific environmental needs occurring as a result of different pathological conditions. The challenging ability of transplanted NPCs to ‘neuroprotect’ the brain from several types of injuries using different and/or articulated bystander strategies is of pivotal importance for the future of stem cell based therapeutic approaches.

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