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Endogenous spinal cord stem cells in multiple sclerosis and its animal model

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ABSTRACT

The adult mammalian spinal cord (SC) harbors neural stem cells (NSCs). The SC-NSCs are mostly quiescent during physiological conditions but are quickly activated in traumatic injury models. The SC-NSCs generate mostly glia, but are able to differentiate into neurons when affected by favourable conditions. An example is the inflammatory milieu in the SC of rat EAE, where the SC-NSCs migrate into demyelinated lesions and give rise to both glia and neurons. In MS, cells with progenitor phenotypes accumulate in inflammatory lesions both in brain and SC, but the extent to which these cells contribute to repair remains to be revealed.

1. Introduction

Inflammatory processes affecting the spinal cord (SC) are common in neurological disease and may cause a serious problem to the patient. Inflammatory lesions in the brain may appear without giving any symptoms, whereas lesions in the SC will always give rise to motor or sensory disturbances and/or affect the bladder function. The most common disease giving rise to these symptoms is multiple sclerosis (MS) which generally gives rise to short central SC lesions whereas neuromyelitis optica, neurosarcoidosis, and systemic lupus erythematosus may cause extensive lesions, see Fig. 1. With time, inflammatory activity in the SC often causes atrophy and permanent neurological disability (Kearney et al., 2015). The acute loss of function can be attributed both to demyelination and/or transaction of neurons but in the acute phase of an inflammatory damage the symptoms are also mediated by blocking of the action potential by inflammatory mediators such as nitric oxide (NO) (Redford et al., 1997). In early MS, the restoration of function after a clinical inflammatory attack is often surprisingly efficient but full resolution can seldom be predicted in the individual case.

The discovery of an adult neural stem cell (NSC) pool has transformed the research of neuroinflammatory/neurodegenerative diseases during the last two decades. The brain stem cells and their niches have been extensively studied both during physiological conditions and in inflammatory models, including experimental autoimmune encephalomyelitis (EAE), the animal model for MS. We know that autoimmune inflammation has a negative impact on the neurogenic niches in the brain, impeding NSC migration and neurogenesis (Pluchino et al., 2008; Tepavcevic et al., 2011). In this review, we have focused on spinal cord neural stem cells (SC-NSCs) and discussed current views in the field both regarding the fate of SC-NSCs during homeostatic conditions and in inflammation, in juxtaposition to brain-NSCs; see Table 1 for an overview of the major discoveries in the field.

2. The identity of spinal cord neural stem cells (SC-NSCs)

The identity of the *bona fide* stem cell in the neurogenic niches of the brain has been attributed to the astrocytic stem cells residing in the subventricular zone (SVZ) of the lateral ventricles (Doetsch et al., 1999) and subgranular zone (SGZ) of the hippocampus (Seri et al., 2001). In the SC, proliferating cells have been reported both in the white and grey matter parenchyma (Horner et al., 2000) and close to the central canal ependymal cell layer (Adrian and Walker, 1962; Johansson et al., 1999; Martens et al., 2002). This has been a matter of controversy for some time since in the brain niches, at least in the SVZ, the ependymal cells did not fulfill the requirements to be identified as NSCs, namely long-term self-renewal and multipotency. In contrast to the ependymal cell layer of the SC, the brain ependymal cells are not proliferating during physiological conditions and have limited self-renewal capacity as they are depleted following injury (Carlen et al., 2009). By using genetic fate mapping it was finally proved that the ependymal layer was the true niche of the SC-NSC, and also the origin of neurosphere-forming ability *in vitro* (Meletis et al., 2008;

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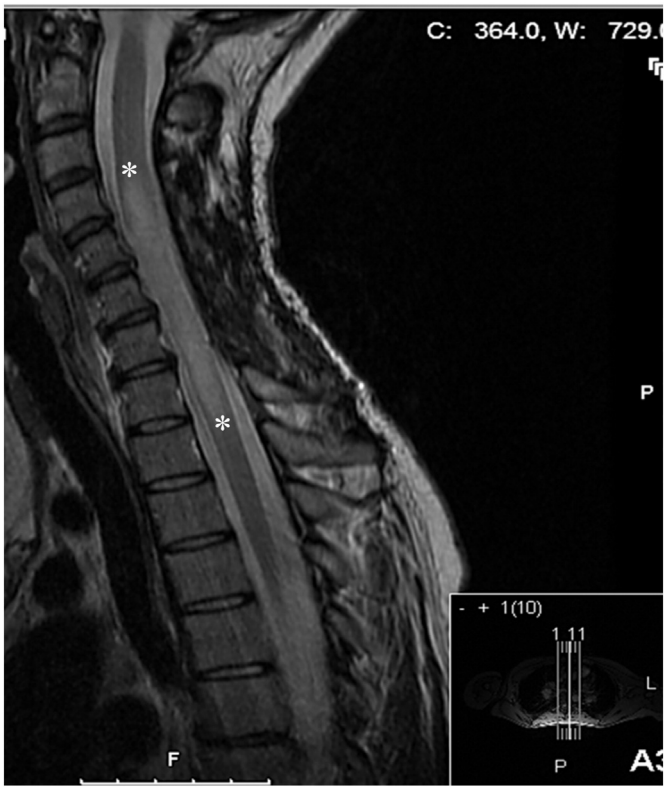

Fig. 1. Magnet resonance imaging (MRI) picture from a patient diagnosed with severe myelitis caused by neurosarcoidosis. The patient was tetraparetic at the time of imaging. Intensive immunosuppression and anti-inflammatory treatment has spared the neurological functions of the patient, who can now live a normal life. Any contribution from progenitor cells to the process of healing is not implied. The figure illustrates an example of severe CNS inflammation in humans. The rostral and caudal borders of the inflammatory lesion are marked with asterisks.

Barnabe-Heider et al., 2010; Pfenninger et al., 2011). In addition, Pfenninger et al. (2011), performed *ex vivo* isolation and analysis of ependymal cells from the SVZ and SC demonstrating that SC-derived ependymal cells have a self-renewing capacity which the brain ependymal cells lacked. Regarding the proliferating cells in the white matter parenchyma, it was demonstrated that they only have limited self-renewal and are of restricted glial lineage (Horner et al., 2000; Barnabe-Heider et al., 2010; Pfenninger et al., 2011). Notably, in the white matter parenchyma, situated in the subpial regions of the SC, there have been reports of a radial glia-like (RG) type of cells (Shibuya et al., 2003; Petit et al., 2011). These cells co-express many NSC-related genes and immune markers, including Sox2, Vimentin and Nestin. In inflammation, the RG-like cells divide and are found in close proximity to Sox2-expressing SC-NSCs, suggestive of a potential regenerative role of these cells during injury (Shibuya et al., 2003; Petit et al., 2011).

3. SC-NSCs are intrinsically different from brain-NSCs

During homeostatic conditions, the SC-NSCs are mostly quiescent displaying low proliferative activity that presumably serves to replenish the ependymal cell layer (Johansson et al., 1999; Pfenninger et al., 2011; Meletis et al., 2008; Barnabe-Heider et al., 2010). This contrasts to the neurogenic niches, the SVZ and the SGZ, where NSCs are dividing to give rise, *via* intermediate cell populations, to new neurons in the olfactory bulb and hippocampus, respectively (Doetsch et al., 1999; Seri et al., 2001). Both brain and SC-NSCs are cultured and propagated successfully *in vitro* using growth factors such as epidermal growth factor (EGF) and/or basic fibroblast (bFGF) growth factor. The culture requirements differ between the brain-derived cultures that are more EGF-dependent and the SC cultures that are bFGF-dependent and can be cultured solely with bFGF, as it is the case in rat (Weiss et al., 1996; Shihabuddin et al., 1997). Upon differentiation, NSCs from both locations generate mostly glia cells, namely astrocytes and oligodendrocytes, while the percentage of neurons is generally low, ranging between 1 and 12%. The proportions of the different mature cell types differ between NSC cultures from the two locations, where SVZ-NSCs generate more neurons than SC-NSCs (Covacu et al., 2014) while the SC-NSCs generate more glia (Covacu et al., 2014; Kulbatski and Tator, 2009). The SC provides a pro-gliogenic environment as both SC-NSCs and pluripotent brain-NSCs generate only glia when transplanted into normal or injured SC (Cao et al., 2001; Shihabuddin et al., 2000). However, the SC-NSCs have the capacity to respond to neurogenic cues by differentiating into neurons when transplanted into the hippocampus (Shihabuddin et al., 2000). In this aspect, the SC-NSCs are similar to niche-derived brain-NSCs that likewise only generate neurons when transplanted into a neurogenic niche (Suhonen et al., 1996).

The SC and brain-NSCs display regional specification and maintain their identity *in vitro*. This regionalization is apparent when the transcriptome of NSCs from the different regions is investigated. In embryonic cells, the SC-NSCs are more enriched in expression of *Hox* genes, while the brain-NSCs show a higher expression of genes such *Lhx2*, *Nr2e1*, *Emx2*, *Otx2* and *FoxG1* (Kelly et al., 2009). In adult NSCs, we also detected an enrichment of *Lhx2*, *Emx2* and *FoxG1* in SVZ-NSCs, which were many hundred fold lower in the SC-NSCs (Covacu et al., 2014). Following differentiation, we identified an enriched neurogenic signature including *Dcx*, *Map2*, *FoxG1*, *Lhx2*, *Nnat* and *Pou3f2* in the SVZ-NSCs which functionally also showed a higher neuronal differentiation compared to SC-NSCs (Covacu et al., 2014). These regionally enriched transcription factors (TFs) influence the activity of other TFs that are universally expressed in NSCs. The region-specific influence of the polycomb factor Bmi-1 on different NSC populations is such an example. Bmi-1 is expressed both in the brain and SC but mediates self-renewal solely in the brain-NSCs. The reason for this is that Bmi-1 is dependent on FoxG1 expression, and thus mediates self-renewal in brain-NSCs, high-expressers of FoxG1, but not in the SC-NSCs where FoxG1 expression is low (Fasano et al., 2009). Similarly, Sox2, a pan-NSC TF, interacts with region-specific TFs. This affects the Sox2 binding to its target-regions and consequently which expression of downstream genes it is going to affect (Hagey et al., 2016).

4. Inflammation effect on NSCs from neurogenic niches

The consensus of most *in vivo* studies is that NSCs are activated by inflammation and respond by changing their proliferation, migration and differentiation pattern or their secretome. Early studies demonstrated the connection between inflammation and disturbances of the neurogenic niche of the SGZ, which no longer supported neuronal differentiation after irradiation and subsequent microglia activation. The impaired neurogenic capacity was related to the niche itself, as progenitor cells isolated from the irradiated brains still could generate neurons *in vitro*. In addition, progenitor cells from non-irradiated brains could not differentiate into neurons when transplanted into irradiated brains. The noxious effect on neurogenesis, mediated by microglia-produced interleukin 6 (IL-6), was counteracted by blocking microglia activation and thereby restoring neurogenesis (Monje et al., 2002; Monje et al., 2003; Ekdahl et al., 2003). Having a negative impact on neurogenesis has been attributed to other inflammatory factors, such as nitric oxide (NO). NO has a regulator function on neurogenesis in the SVZ and OB under normal conditions (Packer et al., 2003; Moreno-Lopez et al., 2004) but at inflammatory levels, it almost completely abrogates neuronal differentiation of SVZ-derived NSCs, *via* upregulation of the TF, REST (Covacu et al., 2006; Bergsland et al., 2014). The picture is complex since other inflammatory factors, such as IFNγ (Wong et al., 2004) or hydrogen peroxide (Perez Estrada et al., 2014) exert a pro-neurogenic effect on NSCs. IFNγ deserves extra attention since it exerts different effects

Table 1
Overview of the major discoveries in the field.

Niche	Species	Condition	Focus/main finding	Reference
SVZ	M	Homeostatic	SVZ-NSC characterization	Doetsch et al. (1999)
SGZ	M	Homeostatic	SVZ-NSC characterization	Seri et al. (2001)
SC	M	Homeostatic/injury	Cell proliferation in SC	Adrian and Walker (1962)
SVZ/SC	M	Homeostatic/injury	Ependymal cell activity	Johansson et al. (1999)
4^{th}V/SC	M	Homeostatic	Proliferation of progenitors in 4thV and SC	Martens et al. (2002)
SC	R	Homeostatic	Proliferation of progenitors in SC	Horner et al. (2000)
SC	M	Homeostatic/SCI	SC-NSC origin and differentiation	Meletis et al. (2008)
SC	M	Homeostatic/SCI	Origin of new glia cells in SC	Barnabe-Heider et al. (2010)
LV	M	Homeostatic/stroke	Ependymal cell characterization	Carlen et al. (2009)
SVZ/SC	M	Homeostatic	Ependymal cell characterization	Pfenninger et al. (2011)
SC	R	SCI	RG characterization	Shibuya et al. (2003)
SC	M	Homeostatic/SCI	RG characterization	Petit et al. (2011)
V/SC	M	Homeostatic	NSC culture from V and SC	Weiss et al. (1996)
SC	R	Homeostatic	NSC culture from SC	Shihabuddin et al. (1997)
SC	R	Homeostatic	SC-NSC differentiation	Kulbatski and Tator (2009)
SVZ/SC	R	Homeostatic/EAE	Brain -NSC *vs* SC-NSC	Covacu et al. (2014)
SGZ	R	Irradiation	Inflammation impairs neurogenesis	Monje et al. (2002)
SGZ	M/R	Irradiation	Inhibition of inflammation restores neurogenesis	Monje et al. (2003)
SGZ	R	LPS infusion/status epilepticus	Activated microglia cells inhibit neurogenesis	Ekdahl et al. (2003)
SGZ	M	EAE	Increased proliferation of NSC	Giannakopoulou et al. (2013)
SVZ	M	EAE	Inhibition of microglia restores NSC function	Rasmussen et al. (2011)
SVZ	M	EAE	Dysfunctional migratory pattern of neuroblasts	Pluchino et al. (2008)
SC	M/H	EAE/MS	Dysregulation of Shh-Gli1 pathway	Wang et al. (2008)
SVZ	M	EAE	Decrease in neuroblast formation increase in pro-oligodendrogenic progenitors	Tepavcevic et al. (2011)
SC	R	EAE	SC-NSC derived glial cells at lesion site	Brundin et al. (2003)
SC	R	EAE	SC-NSC derived neuronal cells at lesion site	Danilov et al. (2006)
SC	R	EAE	Increased neuronal SC-NSC differentiation	Covacu et al. (2014)
SC	R	EAE	Altered SC-NSC function outside lesion sites	Arvidsson et al. (2015)
SC	M	SCI/demyelination/EAE	SC-NSC activation differs between injury models	Lacroix et al. (2014)
SGZ	M	Theiler's virus	Decreased neurogenesis	Jafari et al. (2012)
SVZ	M	Theiler's virus	Increased oligodendrogenesis	Mecha et al. (2013)
SGZ	R	Stroke	Increased neurogenesis	Arvidsson et al. (2002)
SGZ/SVZ	P	Stroke	Increased NSC differentiation	Tonchev et al. (2003)
SVZ	R	Stroke	Increased neurogenesis	Thored et al. (2006)
SVZ	H	Stroke	Increased no. of neuronal progenitors	Macas et al. (2006)

SVZ = subventricular zone; LV = lateral ventricle; V = ventricle; SGZ = subgranular zone; SC = spinal cord; NSC = neural stem cell; RG = radial glia; M = mouse; R = rat; P = primate; H = human; SCI = spinal cord injury; EAE = experimental autoimmune encephalomyelitis.

on the development of neuroinflammation in the brain *versus* the spinal cord, see the review by Pierson et al. (2012).

Severe disturbances of the neurogenic niche integrity have also been observed during experimental autoimmune encephalomyelitis (EAE). Following EAE induction, the SGZ-residing NSCs proliferate but fail to differentiate into neurons (Giannakopoulou et al., 2013). In the SVZ, the NSCs are also induced to proliferate and differentiate, at least initially, but this boost is inhibited during chronic phases (Rasmussen et al., 2011). Interestingly, the SVZ-resident microglia, seem to exhibit a niche-supporting phenotype during the acute phase of EAE. This phenotype subsides during the chronic phase of EAE leaving behind a noxious microglia phenotype (Starossom et al., 2011). This in turn explains why microglia inhibition restores the NSC proliferation and differentiation during the chronic phase of the disease (Rasmussen et al., 2011). The migration of cells from the niche is often dysfunctional and results in an accumulation of neuroblasts in the SVZ without migration to the olfactory bulb (Pluchino et al., 2008). Others have described a decrease in neuroblast formation leading to olfactory deficits in mice (Tepavcevic et al., 2011). On the contrary, the EAE environment seems to be favourable for the pro-oligodendrogenic cell pool in the SVZ (Tepavcevic et al., 2011). Interestingly, the same influence upon the neurogenic and gliogenic cell populations have also been described in susceptible strains with Theyler's virus-induced EAE (Jafari et al., 2012; Mecha et al., 2013). The interplay between inflammation and neurogenesis is however, not crystal clear and could actually result in the promotion of neurogenesis. This is the case in stroke, where neurogenesis is triggered both in the SGZ and SVZ and the newly formed neuroblasts mature into neurons and integrate into a functional neuronal network either in the hippocampus or at the injury site, respectively (Arvidsson et al., 2002; Tonchev et al., 2003; Thored et al., 2006; Macas et al., 2006). By injecting a bacterial compound into the striatum and thereby eliciting an immune reaction without the accompanying tissue damage, Chapman et al. (2015) demonstrated that inflammation, not the injury, plays the pivotal role as neurogenesis-inducer. Even though many of these newly formed neurons do not survive, the mechanism of outside-niche neurogenesis is intriguing and poses valuable leads for the exploration of non-neurogenic niches, such as the SC.

Nevertheless, it is important to note that the studies mentioned above have focused on the integrity of the neurogenic niche, where tightly regulated cues were distorted, resulting in an aberrant NSC behaviour. This does not automatically imply that the NSCs are irreversibly affected. The cells can regain potency and self-renewal capacity after the negative influence is removed (Monje et al., 2002; Pluchino et al., 2008) or migrate towards other cues originating from outside the niche (Picard-Riera et al., 2002). Indeed, SVZ-derived NSCs also migrate into the corpus callosum, striatum, fimbria fornix and cortex both in homeostatic conditions and during demyelinating conditions where they differentiate into immature NG2-expressing cells and mature oligodendrocytes (Menn et al., 2006; Nait-Oumesmar et al., 1999; Picard-Riera et al., 2002; Mecha et al., 2013).

Lastly, the NSCs also have the ability to influence the inflammatory environment or the affected organ through “bystander” action, such as release of trophic factors (Ourednik et al., 2002) or cytokines (Covacu et al., 2009). Transplanted NSCs have been reported to ameliorate EAE (Einstein et al., 2003; Pluchino et al., 2003) and the suggested mechanism was proposed to be immunomodulation of T-cells (Pluchino et al., 2005; Einstein et al., 2007) or of myeloid dendritic cells (Pluchino et al., 2009).

5. Spinal cord stem cells in SCI

Most of the studies exploring the activity of SC-NSCs in inflammation have employed spinal cord injury (SCI) models. The SC-NSCs are contained within the ependymal cell layer which is composed of a heterogeneous cell population out of which some cells are SC-NSCs. During normal conditions, the SC-NSCs rarely divide and only function to replace the ependymal cell pool. In SCI, the SC-NSCs proliferate strongly and migrate towards the injury where they generate mainly astrocytes and oligodendrocytes (Meletis et al., 2008; Barnabe-Heider et al., 2010). The response of the SC-NSCs is imperative to maintain the integrity of the SC outside the injury site since the SC-NSCs contribute to the formation of the astrocytic border of the glial scar (Sabelstrom et al., 2013). Generation of neurons from the endogenous SC-NSC population has not been reported during SCI conditions.

6. Spinal cord stem cells in EAE

Compared to the brain, the SC is the more targeted organ during most EAE models. Undoubtedly, EAE has a tremendous effect on the neurogenic niches in the brain, even in EAE models known to be mainly SC specific. This long-distance effect is probably achieved by factors released in the SC and transported through the glymphatic system to the interstitial space of the brain (Iliff et al., 2012). This can explain how SC-NSCs located far outside inflammatory lesions display the same functional changes as cells located within lesions (Covacu et al., 2014; Arvidsson et al., 2015), and how SCI induce distal effects on the brain neurogenic niches (Yamamoto et al., 2001; Felix et al., 2012).

Nevertheless, it is quite astonishing that we are one of the few groups to study the SC-NSCs during EAE. We use the Dark Agouti (DA) rat EAE model, where we have reported neurogenesis and gliogenesis within inflammatory/demyelinated lesions in the SC. SC-NSCs from the ependymal layer, that had been stereotaxically marked with a lipophilic dye (DiI) incorporated BrdU during the course of EAE and migrated towards lesions where the SC-NSCs matured into O4+ oligodendrocytes, GFAP+ astrocytes (Brundin et al., 2003) or neurons that were either Tuj1+ or NeuN+ positive (Danilov et al., 2006). Whole cell patch clamp measurements showed that these DiI positive, morphologically neuronal-like cells, that had been freshly isolated from EAE lesions, fired overshooting action potentials similar to those of immature neurons (Danilov et al., 2006). In a follow-up study, we further investigated the transcriptome and differentiation pattern of SC-NSCs isolated from EAE and controls (Covacu et al., 2014). The transcriptome analysis revealed an increase in functions associated with neurodegeneration and inflammation and interestingly, many immune-related genes were upregulated in SC-NSC cultures. In contrast, functions associated with nervous system-related genes, many glia-related functions, such as myelination, quantity of Schwann cells, survival and morphology of oligodendrocytes, were decreased in EAE. We could further demonstrate that the population SC-NSCs expressing the pro-neural gene, *Ascl1*, was significantly higher in EAE *vs* control SC-NSCs and upon differentiation this was later matched by a 4–6 fold increase in percentage of neurons (Tuj+) in EAE cultures *versus* controls, see also Fig. 2. Conversely, the EAE-derived cultures generated significantly less (1.5 fold) oligodendrocytes (GalC+) than control SC-NSC cultures. Also, on a transcriptional level, SC-NSCs from EAE had a lower expression level of pro-gliogenic genes such as *Erbb3*, *Nkx6-2* and *Shh*. Interestingly, the Shh pathway has been reported by others to be downregulated in SC-NSCs from a mouse EAE model (Wang et al., 2008). The mechanism behind this change of differentiation pattern has yet to be revealed, however one explanation could be the particularities of the inflammatory environment in the SC (Pierson et al., 2012). Others have reported an EAE-induced diversion from a typical differentiation pattern in the brain-NSCs, where the inflammation has a negative impact on neurogenesis but supports gliogenic differentiation (Tepavcevic et al., 2011). Also, in a model of lysolecithin-induced demyelination, Gad65+/DCX+ neuroblasts from the SVZ were induced to adopt a glial fate (Jablonska et al., 2010).

There are different levels of complexity regarding EAE. The disease severity, duration, clinical and immunological phenotype and target organ affected (brain and/or SC) varies between immunization protocols used and between different species and strains of different model animals used (Pierson et al., 2012). The DA rat-induced EAE, has a relapsing remitting type of disease, with SC-based inflammatory and demyelinated lesions (Storch et al., 1998). The DA rat is very susceptible to disease induction, and compared to, for example mouse models, does not require the use of pertussis toxin or complete Freund's adjuvant. It is possible that the DA rat has a more neurogenesis-permissive SC during EAE, compared to other EAE models. That might be the explanation why Lacroix et al. (2014). did not confirm proliferation or migration of ependymal cells in a mouse model of EAE. There are many questions to be resolved, for example the identity of the EAE-responsive ependymal cells in rat. It would be interesting to investigate why SC-NSCs act so differently in the different EAE models, and if this difference could be attributed to variations in the immune response or in the nature of the SC-NSC population.

7. The human spinal cord

Neurosphere-forming cells with the ability to generate neurons and glia *in vitro* have been obtained from human SC (Dromard et al., 2008; Mothe et al., 2011). Interestingly, these *in vitro*-expanded human SC-derived cells generated mature cell types, including neurons, when transplanted into a SCI model in rats (Mothe et al., 2011). However, the identity of human SC-NSCs and their activity *in vivo* remains to be revealed. Some clues, stemming from comparative studies of primate and human SC central canals, revealed a close resemblance of the cellular composition and organization of the ependymal cell layer between the two species (Alfaro-Cervello et al., 2014). Uni and bi-ciliated ependymal cells in the macaque ependymal layer had proliferative activity and these same kind of cells could be found in the human SC suggesting a similar function there (Alfaro-Cervello et al., 2014). These findings were challenged by a recent study revealing that in most humans beyond the age of 20, the central canal is partly or completely occluded. Also, according to this study, the ependymal cells were rather more closely related to ependymomas, lining blood vessels, something that has been misinterpreted as a central canal lumen (Garcia-Ovejero et al., 2015). Nevertheless, when a central canal was present and had a typical cellular organization, the ependymal cells seemed to be able to react and possibly proliferate in response to trauma (Cawsey et al., 2015). Thus, at least in some individuals or in some portions of their SC, there is a proliferative activity reminiscent of SC-NSCs. In this context it is important to mention the filum terminale (FT), which is a structure at the most terminal part of the SC, providing longitudinal support by anchoring the SC to the coccyx. This structure harbors cells that express NSC-related markers such as Sox2 and Musashi1 and are located in an islet-like manner along a central canal-like cavity. Also, cultured FT-derived cells form neurospheres and differentiate *in vitro* into glia and neurons, both demonstrated by immunocytochemistry and patch clamp measurements where the cells displayed the typical electrophysiological properties of differentiated cells (Varghese et al., 2009; Arvidsson et al., 2011). When transplanted into a stroke injury model, these cells migrated into the lesioned CA1 region of the hippocampus and differentiated (Varghese et al., 2009).

Finally, studies on human brain or SC from MS patients are limited to just a few. Immunohistochemistry analysis of SVZ from MS patients revealed an accumulation of a polysialylated form of neural cell adhesion molecule (PSA-NCAM) positive progenitor cells that showed signs of proliferation. These cells could also be found in demyelinated lesions and chronic active lesions closer to the SVZ, expressing pro-gliogenic markers such as Sox9, Sox 10 and Olig2 (Nait-Oumesmar et al., 2007). Moreover, similarly to the animal studies, the number of DCX+

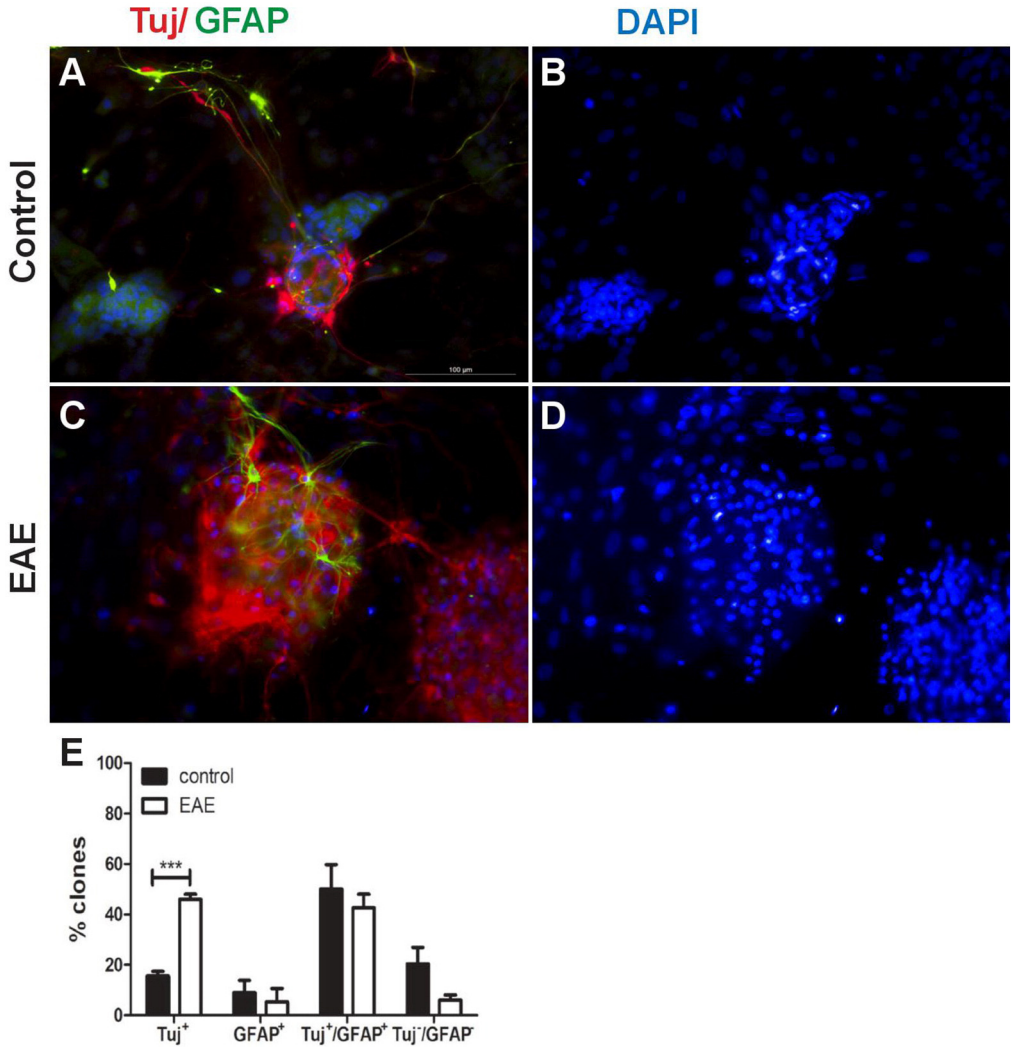

Fig. 2. SC-NSCs from EAE animals generate significantly higher percentage of neuronal (Tuj+) positive neurospheres than SC-NSCs from control animals. Panels A–D show representative pictures of neurospheres cultured from SC of control animals (A, B) and EAE animals (C, D) immune-labelled for neurons (Tuj) or astrocytes (GFAP) (A, C) and DAPI was used to stain nuclei (B, D). The neurospheres were counted and divided into four groups dependent on their differentiation pattern: single competent (either GFAP+ or Tuj+) bi-competent (Tuj+/GFAP+) or double-negative for both Tuj and GFAP. Three glasses per conditions were counted; $n = 3$; bars show mean ± SEM; unpaired *t*-test, two tailed, $^{***}p < 0$. Unit bar = 100 μm.

neuroblasts was severely decreased in MS SVZ (Tepavcevic et al., 2011). Lesions in both brain and SC from MS patients were also found to have an accumulation of Nestin positive progenitor cells out of which the majority were also Musashi1 positive. In addition, some of these cells expressed the cell proliferation marker Ki67 and early glial or neuronal markers (Snethen et al., 2008). This suggests that progenitor cells, possibly NSCs, are activated during MS at different locations in the CNS, and might be responsible for the regeneration seen in the remission phases of this disease.

8. Concluding remarks

The SC-NSCs constitute a yet still uncharted area in the research field of regenerative medicine. The SC-NSC niche has been pinpointed to the ependymal layer lining the central canal, at least in experimental animals. The cells display regional characteristics and differ in their transcriptome from the brain-NSCs. Even though they are mostly quiescent and mainly generate glia cells in injury models, the SC-NSCs demonstrate multipotency and also generate neurons when transplanted into the right conditions. The SC-NSCs respond strongly to traumatic injuries, however, the identity of the SC-NSCs that are active during physiological *versus* inflammatory conditions remains to be revealed. There is also some controversy related to their activity in other inflammatory/injury models. Studies from the rat EAE, and especially the DA rat, provide evidence for SC-NSC response in this condition, resulting in their differentiation at the lesion site. Also in human MS lesions, there is some evidence for accumulation of progenitor cells both in the brain and remarkably, also in the SC. More in-depth studies are needed to understand the particularities of the SC-NSCs and evaluate their usability in treatment of neuroinflammatory diseases.

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