Mol Neurobiol (2018) 55:3152–3171
DOI 10.1007/s12035-017-0566-7

Neural Stem Cell-Based Regenerative Approaches for the Treatment of Multiple Sclerosis

Juan Xiao[1,2] · Rongbing Yang[2] · Sangita Biswas[3,4] · Yunhua Zhu[1] · Xin Qin[1] · Min Zhang[1] · Lihong Zhai[1] · Yi Luo[1] · Xiaoming He[1] · Chun Mao[1] · Wenbin Deng[3,4]

Received: 27 February 2017 / Accepted: 19 April 2017 / Published online: 2 May 2017

Abstract Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory, and demyelinating disorder of the central nervous system (CNS), which ultimately leads to axonal loss and permanent neurological disability. Current treatments for MS are largely comprised of medications that are either immunomodulatory or immunosuppressive and are aimed at reducing the frequency and intensity of relapses. Neural stem cells (NSCs) in the adult brain can differentiate into oligodendrocytes in a context-specific manner and are shown to be involved in the remyelination in these patients. NSCs may exert their beneficial effects not only through oligodendrocyte replacement but also by providing trophic support and immunomodulation, a phenomenon now known as "therapeutic plasticity." In this review, we first provided an update on the current knowledge regarding MS pathogenesis and the role of immune cells, microglia, and oligodendrocytes in MS disease progression. Next, we reviewed the current progress on research aimed toward stimulating endogenous NSC proliferation and differentiation to oligodendrocytes in vivo and in animal models of demyelination. In addition, we explored the neuroprotective and immunomodulatory effects of transplanted exogenous NSCs on T cell activation, microglial activation, and endogenous remyelination and their effects on the pathological process and prognosis in animal models of MS. Finally, we examined various protocols to generate genetically engineered NSCs as a potential therapy for MS. Overall, this review highlights the studies involving the immunomodulatory, neurotrophic, and regenerative effects of NSCs and novel methods aiming at stimulating the potential of NSCs for the treatment of MS.

Keywords Neural stem cell · Neural progenitor cell · Microglia · Oligodendrocyte · Multiple sclerosis

Introduction

Multiple sclerosis (MS) is one of the most common neurological disorders of the central nervous system (CNS) in young adults. The pathological hallmarks of the disease are the appearance of multifocal inflammatory lesions in the CNS separated in time and space, demyelination, and axonal transaction [1, 2]. Relapsing-remitting multiple sclerosis (RRMS) is the most common form of MS and has a biphasic disease course marked by alternating episodes of acute neurological deficits and/or worsening of a given neurological function (i.e., relapse), followed by a complete or partial recovery (i.e., remission). Generally after 15–25 years, ~70% of the RRMS patients develop secondary progressive MS (SPMS) which is characterized by progressive neurological decline independent of relapses (inflammation) [3]. Around 10–15% of the MS patients present primary progressive disease (PPMS)

Juan Xiao and Rongbing Yang contributed equally to this work.

✉ Sangita Biswas
sangita.biswas@ucdmc.ucdavis.edu

✉ Wenbin Deng
wbdeng@ucdavis.edu

[1] Department of Neurology, Xiang Yang Central Hospital, Medical College of Hubei University of Arts and Science, Xiangyang, Hubei, China

[2] Department of Biological Treatment, Handan Central Hospital, Handan, Hebei, China

[3] School of Pharmaceutical Sciences, Sun Yat-sen University, Shenzhen, Guangdong, China

[4] Department of Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis, 2425 Stockton Boulevard, Sacramento, CA 95817, USA

characterized by the steady progressive deterioration in neurological function from the onset of symptoms, without preceding or concomitant relapses [4].

Etiology and Pathology of MS

MS is an immune-mediated disease in which the body's immune system mistakenly attacks myelin in the CNS. Apart from the major histocompatibility complex (MHC) loci, many other non-MHC genetic variants involved in MS pathogenesis have been recently identified [5]. Notably, broad complex-tramtrack-bric-a-brac (BTB) and Cap'n'collar (CNC) Homology 1 basic leucine zipper transcription factor 2 (BACH2), which is required for efficient formation of regulatory T (Treg) cells, are found to be downregulated in blood cells of MS patients compared to healthy subjects, which may be responsible for the impaired Treg functions in MS patients [6]. Treg cells have been recognized as the critical immunomodulators of the adaptive immune system in MS. Deletion of Treg cells causes spontaneous autoimmune disease in mice, whereas augmentation of Treg cell function can prevent the development of or attenuate the signs in the experimental autoimmune encephalomyelitis (EAE), the animal model of MS [7]. MS is also associated with impaired maturation of Treg cells [8]. Remission in RRMS has been shown to correspond with increased proportions of FoxP3+ Treg cells in the blood [9]. Thus, Treg cells are being considered as potential therapeutic targets in MS [10, 11].

Several environmental candidates such as nicotine smoking, low serum vitamin D levels [12, 13], and viral infection were found to increase the risk of developing MS, by inhibition of mitochondrial respiratory chain in the CNS and contributing to demyelination [14], activation of potentially encephalitogenic T cells and their trafficking to the CNS [15], and increased production of proinflammatory cytokine interleukin-6 (IL-6) [16]. Loss of self-tolerance may be triggered by an environmental antigen, virus, or other factors discussed above [17]. Epstein-Barr virus (EBV) [18] and human herpes virus (HHV)-6 [19] have been consistently linked with MS pathogenesis, and 99% of MS patients are EBV seropositive [20]. The adoptive transfer of in vitro-expanded autologous EBV-specific CD8+ T cells into patients with severe SPMS could reduce disease activity and decrease intrathecal immunoglobulin production of EBV-infected autoreactive B cells [21].

Immunopathology of MS

Two model theories of lesion development in MS have proposed: the outside-in model and the inside-out model [22]. In the outside-in model, MS lesions develop from the outside (myelin) to the inside (axons); in the inside-out model, the lesions develop from the inside (axons) to the outside (myelin). The outside-in model refers to a primary CNS demyelination, usually induced by anti-myelin autoimmune cells generated in the periphery, while the inside-out model refers to a primary CNS axonal degeneration and subsequent recruitment of systemic/adaptive immune cells [23, 24].

Denuded axons are vulnerable and start degenerating as the disease progresses [25, 26]. Despite the extensive axonal loss in acute MS lesions, relapses are reversible by the potent compensatory mechanisms in the brain [27, 28]. The conversion of RRMS to SPMS is thought to occur when the brain exhausts its capacity to compensate for further axonal loss [29, 30]. Chronically demyelinated axons have an increased energy requirement to maintain conduction velocity in the absence of myelin [31, 32]. Mitochondrial density and activity were increased within demyelinated axons in MS lesions which coincided with increased oxidative stress [33, 34].

Remyelination Failure in MS

Remyelination is the regenerative process by which demyelinated axons are reinvested with new myelin sheaths. Spontaneous and robust remyelination occurs at the early stages of MS [35], occurring within a month or two after active demyelination [36]. Experimental animal models of CNS demyelination indicate remyelination is not performed by pre-existing mature oligodendrocytes [37], but involves new remyelinating oligodendrocytes derived from the maturation of quiescent oligodendrocyte progenitor cells (OPCs) distributed throughout the adult CNS [38, 39]. In the corpus callosum, remyelinating oligodendrocytes can also be derived from neural stem and precursor cells of the adult subventricular zone as shown in animal models [40, 41]. Moreover, it has also been observed that both the numbers and the differentiation stages of OPCs and mature oligodendrocytes are highly variable within lesions of different patients and in different lesion stages [42].

The eventual failure of remyelination that occurs as MS progresses results from multiple factors such as the generation of a nonpermissive environment which prevents OPC differentiation, and also from a slowly progressive loss of the OPC pool from established lesions [43, 44].

Parenchymal OPCs are mostly responsible for oligodendrogenesis and remyelination in MS [45]. These OPCs are present in robust densities inside the lesions during early phases of MS pathology [46], although in chronic MS lesions, their number becomes significantly lower [47, 48].

Within a demyelinating lesion, activated CD4+ and CD8+ T cells, as well as macrophages, are thought to act in concert with reactive microglia to release a milieu of proinflammatory factors that lead to oligodendrocyte dysregulation and apoptosis [49]. Oligodendrocytes are particularly vulnerable to

antigen recognition and cytotoxicity by CD8+ cytotoxic T lymphocytes since they express MHC class I antigens under certain inflammatory conditions [50, 51]. Postmortem study of the brain tissue from some RRMS patients revealed that very early MS lesions exhibit extensive oligodendrocyte apoptosis in myelinated tissue containing few or no lymphocytes [52], which raises the possibility of nonimmune-related toxic effects directly against the oligodendrocytes. Oxidative damage is another common contributor to oligodendrocyte loss under many pathological conditions like MS [53, 54].

Current Limitations of the Disease-Modifying Treatments for MS

Currently available treatments for MS primarily target the underlying immunologic etiology of the disease [55]. While significantly effective in preventing the frequency of relapses, these treatment options have little benefit for SPMS patients since they do not prevent the continuous axon loss and progression and irreversible disability. Secondly, a shift from adaptive to innate immunity characterized by abnormalities of dendritic cells' (DCs) activation or maturation may underlie the transition to the progressive phase of the disease [56]. Current immunomodulatory drugs are directed primarily against the cells and mediators of the adaptive immune system [57]. Thus, preventing this transition, perhaps by acting at the level of the innate immune system, is an important therapeutic strategy.

Development of therapies to benefit progressive MS patients will require a more comprehensive understanding of the pathogenesis of progressive MS. It is suggested that during the late stages of the disease, the inflammation is relatively less, but the susceptibility of the target tissue to neurodegeneration and axonal degeneration increases [1]. Therefore, we argue that an essential strategy for MS therapy is to target the axonal pathology aiming for neuroprotective as well as neuroregenerative outcomes.

Models to Study MS Pathology

Various animal models such as T cell-mediated (EAE), toxin- or virus-induced demyelination, and genetic models of demyelination are now used to understand the pathological and etiological aspects of MS.

EAE offers a practical strategy for reproducing certain distinct adaptive immune-mediated pathologic features of demyelination. EAE shares many pathological features with MS including chronic neuroinflammation, multifocal autoimmune demyelination, and axonal loss and is triggered by an autoimmune attack on the CNS [58].

Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease (TMEV-IDD) is the most widely studied virally induced demyelinating disease (in mice) which can be explained by the inside-out model [59]. Following TMEV infection, axonal degeneration precedes demyelination [60]. In this model, mice develop chronic progressive demyelinating disease without remission, similar to the disease course of PPMS. Epidemiological studies suggest that viral models are useful in understanding the possible viral etiology [61], the process of the axonal injury/repair in MS [62], and the interplay between genetic predisposition and environmental insults [26]. It is also important to evaluate the therapeutic potential of engrafted neural stem cells (NSCs) in the presence of a persistent viral infection that is associated with chronic neuroinflammation and demyelination [63].

Cuprizone-induced demyelination model is a useful model of noninflammatory demyelination which acts as a preclinical tool for screening candidate drugs for remyelination-promoting effects. Also, focal injection of lysolecithin into the spinal cord white matter of mice produces a discrete demyelinating lesion followed by spontaneous and complete remyelination [64].

Animal models that enable the study of remyelination in the presence of ongoing inflammation are needed to examine whether current or new therapies can promote remyelination in the face of the inhibitory cues present in the MS plaque microenvironment. An innovative animal model combines cuprizone-induced demyelination with the transfer of myelin-reactive T helper 17 (Th17) cells which delays the endogenous repair process. The IFN-/IL-17-secreting T cells in the corpus callosum extend the period of demyelination and open the window to test the beneficial effects of available putative remyelinating therapies [65]. Recently, it has been shown that cerebrospinal fluid from SPMS patients injected in mice could induce inflammatory demyelination, axonal loss, and astrogliosis [66].

All the models mentioned above mimic only a part of MS pathology, and they act in a complementary way. Treatments should be assessed in multiple models to reflect their various aspects on adaptive and innate immune systems, demyelination and remyelination, short-term effect, and long-term prognosis. For example, interferon-β (IFN-β) could alleviate inflammation and reduce demyelination in EAE models. However, in cuprizone-treated mice, IFN-β exerts side effects regarding remyelination in the absence of an immune-mediated demyelination, which questions their long-term use as a possible MS treatment [32].

Participation of Endogenous NSCs in Remyelination: Studies in Animal Models of MS

In the last decade, growing interest has focused on utilizing NSCs to promote remyelination. In the adult CNS, tissue-specific germinal niches, such as the subventricular

zone (SVZ) of the lateral ventricles and the subgranular zone of the dentate gyrus (DG) of the hippocampus, contain multipotent NSCs with the capacity to self-renew and differentiate into functional neurons and glia [57, 67]. Multipotent NSCs have also been isolated from a subcortical white matter of the adult human brain [68]. A recent study revealed the existence of dormant ependymal CD133+ NSCs lining the surface of the fourth ventricle in mice which could be mitotically activated and differentiated into neurons and glia upon stimulation [69, 70].

NSCs in the adult mammalian brain have been shown to give rise to rapidly dividing neural progenitor cells (NPCs) to produce neurons, astrocytes, and oligodendrocytes, and functionally contribute to (although modest) cognition and repair processes after injury [69, 71]. For example, neuroblasts in the adult mice SVZ can be primarily directed to an oligodendrocyte fate upon lysolecithin-induced demyelination of the corpus callosum [72, 73]. In EAE, NSCs can become activated, migrate to the lesions, and differentiate into oligodendrocytes, providing another source of myelinating oligodendrocytes [68, 74]. Retroviral-mediated Mash1/Ascl1 misexpression redirects neurogenic intermediate progenitors to an exclusive oligodendrocyte lineage in the adult subgranular zone (SGZ) [71, 75]. It is now believed that radial glia cells not only serve as progenitors for many neurons and glial cells soon after birth, but also give rise to adult SVZ stem cells that continue to produce astrocytes, neurons [71, 76], and, to a lesser extent, oligodendrocytes [77]. Neurogenic capacity is disrupted during aging, while the ability to produce new oligodendrocytes is not compromised in the human brain [78]. In the aged SVZ, proliferation is reduced due to loss of stem cell numbers, inability to self-renew, or increases in cell cycle length [79]. The remaining actively proliferating NSCs in SVZ and DG decrease over time in the aged brain, transforming into astrocytes [80, 81].

The participation of SVZ-derived progenitors in remyelination has been demonstrated in several experimental mouse models of demyelination [82, 83]. Acute EAE results in enhanced migration of SVZ-derived NPCs to the olfactory bulb and triggers their mobilization in the periventricular white matter. The mobilized cells give rise to oligodendrocytes in the inflammatory demyelinating lesioned white matter to replace the dysfunctional or dying oligodendrocytes [74]. In contrast, during the chronic/nonremitting phase of EAE (analogous to the progressive form of MS), NSC and NPC proliferation is attenuated in the SVZ and hippocampus [84].

In the TMEV-IDD model in mice, progenitors in the SVZ are mobilized to undergo oligodendrogenesis and migrate toward demyelinated areas close to the lateral ventricles in the corpus callosum to participate in remyelination [40].

In the cuprizone-induced demyelination model, large numbers of NPCs were shown to migrate into the corpus callosum where the majority of these cells differentiated into oligodendrocytes and exhibited robust capacity to remyelinate, especially in the rostral regions adjacent to the SVZ. These NPC-derived oligodendrocytes reestablished the nodes of Ranvier and g-ratios, and newly formed myelin was equivalent to those of healthy control mice [41]. However, in a chronic model when demyelination is sustained over a period of time (after long-term cuprizone administration), SVZ-derived NPCs minimally contribute to myelin repair [84]. This is associated with an exhaustion of the pool of SVZ progenitors which have a limited self-renewal potential [85], a drastic drop of their proliferation and mitochondrial dysfunction in NPCs [86].

The NPCs and OPCs play a key role in augmenting the endogenous myelin/neuronal repair capacity in MS-like disease, likely via CXCL12/CXCR4 autocrine signaling post inflammation [87]. Generally, CNS inflammation in MS patients is associated with upregulation of the chemokine ligand CXCL12 expression. In EAE mice, CXCL12 expression in the DG and corpus callosum was persistently increased following spontaneous recovery even though CNS inflammation had subsided, and the numbers of NPCs in both regions increased correspondingly. A significant portion of the NPCs and OPCs express the CXCL12 and CXCL12 receptor CXCR4. Thus, the increased levels of CXCL12 expression in the DG and corpus callosum of EAE-recovering mice may be associated with the promotion of neuro/oligodendrogenesis generating CXCR4+ CXCL12+ NPCs and OPCs endowed with intrinsic neuro/oligondendroglial differentiation potential.

Therapeutic strategies utilizing endogenous NSCs have great potential since it avoids the intricate procedure of generating exogenous generation of NSCs which involves lengthy differentiation protocols [88]. Currently, available drugs and recombinant cytokines or soluble factors need an intensive study to exploit their potential in booting endogenous remyelination.

Vitamin D_3 may directly enhance proliferation of NSCs and their differentiation into neurons and oligodendrocytes in EAE mice. NSCs constitutively expressing the vitamin D receptor (VDR) exhibited increased expression of neurotrophic factors neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) after exposure to vitamin D_3 [89]. Increased remyelination in the hippocampus by endogenous progenitor cells was observed in rats receiving vitamin D_3 following ethidium bromide (EB)-induced demyelination [90]. 1,25-Dihydroxyvitamin D_3 (1,25(OH)2D3) has an immunomodulatory effect and has been implicated in the pathogenesis of MS. There are several additional benefits to administering vitamin D. Vitamin D_3 induces human DCs to adopt a tolerogenic phenotype, characterized by decreased expression of CD40, CD80, and CD86; low interleukin-12 (IL-12) release; and enhanced anti-inflammatory interleukin-10 (IL-10) secretion [91]. It also reduces the serum levels of pathogenic IL-17 in RRMS patients [92].

Limitation of Endogenous NSC Toward Remyelination

In general, the microenvironment at and around the lesion site during demyelination appears to favor astrogliogenesis rather than oligodendrogenesis from SVZ-derived cells. This has been evidenced in several studies. For example, epidermal growth factor (EGF) plays a dual role in MS and EAE. In the lysolecithin-induced demyelination model, intravenous (i.v.) infusion of EGF dramatically promoted the proliferation and migration of SVZ NSCs as well as their differentiation into oligodendrocytes in the corpus callosum [93]. However, in chronic MS lesions, EGF signaling is associated with astrogliosis and glial scar formation. In fact, EGF was shown to play a pivotal role in astrogliogenesis at the expense of oligodendrogenesis [94]. Interestingly, EAE mice injected (i.v.) with anti-EGF neutralizing antibody at day 9 after the initial proliferation phase of SVZ-derived NSCs had significantly ameliorated EAE symptoms via induction of neurogenesis and oligodendrogenesis in the SVZ [95]. Similarly, an upregulation of bone morphogenetic protein 4 (BMP4) protein levels is usually detected during active demyelination, and NSCs treated with BMP4 produced more astrocytes in vitro. Intraventricular infusion of Noggin, an endogenous antagonist of BMP4, increased the number of Olig2-positive oligodendrocytes and decreased astrocyte numbers in the SVZ after cuprizone-induced demyelination in mice [96].

Fingolimod (FTY720) is a sphingosine-1-phosphate (S1P) receptor modulator, and the first oral treatment option available for RRMS [97]. However, FTY720 did not promote remyelination in lysolecithin-induced demyelination animal models [98]. Administration of FTY720 to JHM strain of mouse hepatitis virus (JHMV)-infected mice resulted in enhanced migration and increased proliferation of transplanted NPCs after spinal cord engraftment, yet failed to improve disease or increase remyelination [99].

Treatment with IL-4 and IL-10 upregulated the surface adhesion molecule lymphocyte function-associated antigen 1 (LFA-1) and chemokine receptors CXCR4 on NSCs, thus facilitating migration of NSCs toward the CNS inflammatory foci [100]. Overall, it is apparent that stimulation of endogenous NSCs with beneficial factors is a promising approach for the treatment of MS and requires further research to reveal its therapeutic potential and the timing, dose, and safety of each candidate. However, NSC-derived oligodendrogenesis is limited compared to astrogliogenesis.

NSCs-Microglia Cross Talk: Effect on NSC Survival and Differentiation, and Immunomodulation

Microglia, the resident macrophages in the CNS parenchyma, are a heterogeneous group of monocyte-derived cells serving multiple roles within the brain [101]. They have been actively involved in MS pathogenesis both in early as well as in late stages of MS lesions formation [102]. Intrinsic triggers such as subtle pathological changes in the CNS induce the formation of clusters of activated microglia [103], which adopt a cytotoxic phenotype when exposed to proinflammatory molecules by releasing reactive oxygen species (ROS) and nitric oxide (NO) [104]. This further aggravates the imbalance between increased energy demand and decreased energy supply in chronically demyelinated axons [105].

Phagocytosis and removal of damaged myelin seem to be the major roles of microglia in MS, and removal of myelin debris is a prerequisite of successful remyelination [106]. In response to inflammation and infection in the CNS, oligodendrocytes release cytokines that recruit microglia to phagocytosis inhibitory molecules present in the lesion microenvironment [107], thereby aiding repair and regeneration [108]. Inactive lesions in SPMS comprised an external border of activated microglia. Impaired phagocytosis of myelin fragments on the surface of microglia was in part responsible for the failure of remyelination [109] (Fig. 1).

Microglia are also important modulators of the inflammatory milieu in the CNS in MS [110]. During the active phase of the MS, activated microglia produce proinflammatory mediators [111], chemokines, and oxidizing radicals which are potentially detrimental to oligodendrocytes, suggesting a correlation between microglial activity and oligodendrocyte damage in MS [105]. Resident microglia can establish a cross talk with infiltrated immune cells, including IL-17+ γδ T cells, regulating their recruitment, activation, and function within the CNS [112, 113]. 18β-glycyrrhetinic acid (GRA) effectively reduced CNS inflammation and myelin damage in EAE in C57BL/6 mice through inhibition of microglia activation via the suppression of mitogen-activated protein kinase (MAPK) signal pathway which plays an important role in the interferon gamma (IFN-γ)-induced expression of proinflammatory genes in activated microglia. GRA-modulated microglia downregulated the production of proinflammatory cytokines and chemokines, which reduced the recruitment of encephalitogenic T cells into the CNS [114], and promoted remyelination [115].

NSC Survival and Differentiation

Microglia are thought to play a role in the migration of NSCs, as well as in effecting their survival and differentiation. In both acute and chronic EAE, microglia number was significantly higher in CNS regions containing transplanted NPCs [116]. Soluble factors released from mouse microglial cells direct the migration of NPCs in vitro and in vivo [117]. In the EAE brain, microglia produce stromal cell-derived factor-1 (SDF-1), monocyte chemoattractant protein-1 (MCP-1), and hepatocyte growth factor (HGF), accounting for the inflammation-induced attraction of transplanted NPCs (which

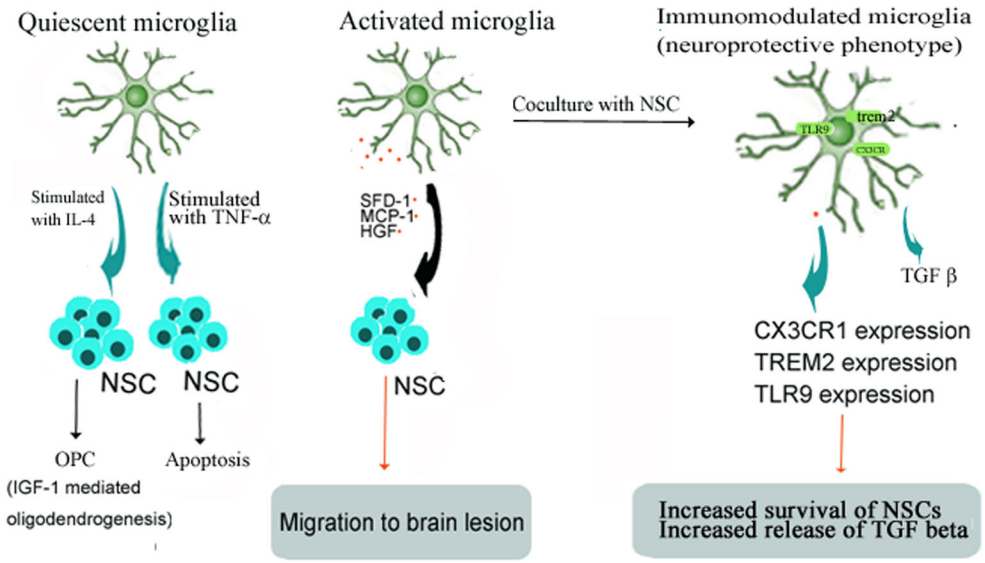

Fig. 1 NSC survival, differentiation, and immunomodulation are shaped by NSC-microglia cross talk. Microglial-derived signals determine NSC survival and differentiation in EAE. Conversely, NSC-derived signals cause immunomodulation in microglia via paracrine factors and signaling pathways. Resting microglia, stimulated by IL-4 in vitro, promotes insulin-like growth factor-1 (IGF-1)-mediated oligodendrogenesis from adult NPCs in mice [120]. On the other hand, microglia-derived tumor necrosis factor-alpha (TNF-α) induced the expression of the BH3 (Bcl-2 homology domain-3) in NPCs by an NF-kB (nuclear factor-kB)-dependent mechanism and increased NPC apoptosis by a mitochondrial pathway [121]. Soluble factors released from mouse microglial cells direct the migration of NPCs in vitro and in vivo [117]. In the EAE brain, microglia produce stromal cell-derived factor-1 (SDF-1), monocyte chemoattractant protein-1 (MCP-1), and hepatocyte growth factor (HGF), responsible for the inflammation-induced attraction of transplanted NPCs into white matter lesions [118]. In an allogeneic co-culture model, both human NPCs and microglia showed increased survival and proliferation, and the release of transforming growth factor-β (TGF-β) was also upregulated. NSCs can induce a significant upregulation of the surface molecules CX3CR1 on microglia which is associated with a neuroprotective phenotype, and triggering receptor expressed on myeloid cells-2 (TREM2) [119, 122–124]

constitutively expressed cognate receptors for these chemokines) into white matter tracts [118]. In an allogeneic co-culture model, both human NPCs and microglia showed increased survival and proliferation, and the release of transforming growth factor-β (TGF-β) was also upregulated. However, differentiations of NPCs were hindered by microglia [119]. Depleting microglia from hippocampal cultures reduce NSC survival and proliferation. Microglia, stimulated by IL-4 in vitro, encouraged insulin-like growth factor-1 (IGF-1)-mediated oligodendrogenesis from adult NPCs in mice [120]. On the other hand, microglia-derived tumor necrosis factor-alpha (TNF-α) induced the expression of the BH3 (Bcl-2 homology domain-3) only family member Puma in NPCs by an NF-kB (nuclear factor-kB)-dependent mechanism and increases NPC apoptosis by a mitochondrial pathway [121].

NSC-Induced Modulation of Microglial Function

Novel treatment strategies should utilize NSCs to modulate host microglial phenotypes and functions to benefit neuroprotection and repair. NSCs or NPCs may not only be shaped by microglia but also they, in turn, are capable of manipulating microglia functions and activity. NSCs can transform microglia from a harmful to a neuroprotective phenotype by significantly increasing the expression of molecules associated with a neuroprotective phenotype in adult mouse brain [122]. For example, NSCs can induce a significant upregulation of the surface molecules CX3CR1 on microglia which is associated with a neuroprotective phenotype [123] and triggering receptor expressed on myeloid cells-2 (TREM2) [124]. Injection of primary mouse NPCs into the striatum of C57BL/6 mice causes a significant increase in an absolute number of Iba-1+ microglia with activated morphology, and those effects were mainly exerted through vascular endothelial growth factor (VEGF), which is secreted by grafted NPCs in significant amounts [125].

NSCs have been shown to improve host neuronal viability in mouse organotypic brain slice cultures by switching microglia from a detrimental to a neuroprotective phenotype, through the microglial Toll-like receptor 9 (TLR9)-extracellular-regulated protein kinases 1/2 (ERK1/2) pathway. These beneficial modulatory effects of NSCs were abrogated by the microglial inhibitor minocycline [122]. NSCs that were preconditioned with minocycline in vitro before transplantation had upregulated expression of Nrf2-regulated antioxidant genes, and enhanced the survival of grafted cells and release of paracrine mediators, such as BDNF and VEGF [126]. Conversely, microglial activation improved regenerative potential in the SVZ in the chronic phase of EAE. In vivo

treatment with minocycline increased NSC proliferation and their differentiation into mature oligodendrocytes in the SVZ by inhibiting the activation of microglia [127].

Tissue and Cellular Sources for NSCs: Utility and Limitations

Various cell types may serve as a source of NSCs or NPCs, for example, human embryonic stem (ES) cells (hESCs) [128], fetal and adult brain SVZ cells, and postmortem human CNS tissue [129]. Autologous mesenchymal stem cells (MSCs) are another source of neural stem cells for MS because they are readily obtained from adult bone marrow (BM) [130]. Experiments showed that the therapeutic effects of bone marrow-derived NSCs (BM-NSCs) and SVZ-NSCs were almost identical in EAE models, and BM-NSCs also exhibited comparable morphological properties and possess a similar ability to differentiate into neurons, astrocytes, and oligodendrocytes both in vitro and in vivo [131].

The generation of induced pluripotent stem cells (iPSCs) from adult skin fibroblasts has heralded the possibility of autologous transplants that would circumvent histocompatibility barriers and ethical problems [132]. iPSCs can differentiate efficiently into NSCs and, subsequently, into specific neural lineages [133]. The gene expression profiles of iPSCs derived NSCs are comparable to those of human fetal-derived NSCs and these iPSCs-NSCs could be differentiated into neurons, astrocytes, and oligodendrocytes [134]. A research group used Sendai virus constructs encoding four iPSC transcriptional factors (Sox2, Oct4, Klf4, and c-Myc) to derive neural stem cells from CD34+ cells from both cord blood cells and adult peripheral blood [135]. Experiments demonstrated that mouse iPSCs-derived NPCs (miPSCs-NPCs) differentiated into mature oligodendrocytes in demyelinated *Shiverer* mice and generated compact myelin around host axons and restored nodes of Ranvier and conduction velocity as efficiently as CNS-derived NPCs [136].

However, several aspects of human iPSCs may be impacted by epigenetic mechanisms. A recent study demonstrated that human iPSC-derived NPCs from patients with schizophrenia (SZ) had perturbations in canonical WNT signaling, which may be caused in part by increased oxidative stress within the nervous systems commonly observed in MS patients [137]. NPCs differentiated from iPSCs that collected from blood samples of PPMS patients provided no neuroprotection against active CNS demyelination compared to NPCs from control iPSC lines [138].

Several recent reports indicate that NSCs and NPCs can be directly generated from skin fibroblasts by direct reprogramming [139]. Plasmid vectors containing the EBV-derived oriP/EBNA1 defined expression factors and a small hairpin directed against p53 could reprogram adult human fibroblasts to induced NSCs (iNSCs) without the addition of small molecules [140]. Direct conversion of somatic cells into stably expandable iNSCs and induced NPCs (iNPCs) may prove to be highly efficient, safe, and labor-saving, compared with the circuitous two-step strategy used during the conversion of somatic cells to iPSCs and subsequent differentiation into neural stem cells [141]. iNPCs could be induced directly from human fibroblasts by overexpression of SRY-box 2 (SOX2) protein in combination with a chemical cocktail under 3D sphere culture conditions [142]. Highly expandable human NSCs with multipotent neural differentiation potential can also be directly generated from human fibroblasts by lentiviral transduction with four to five reprogramming genes [143].

Mouse fibroblast-derived tripotent iNSCs could be differentiated not only into neurons and astrocytes but also into oligodendrocytes capable of integration into dysmyelinated *Shiverer* brain [144]. Future experiments will be necessary to help define the potential of these cells in the context of inflammation and their tissue tropism in MS. The therapeutic potential of human NPCs may differ greatly depending on the method of derivation and expansion [145]. The expression of neurotrophic factors in NPCs usually decreases with time in culture [146], and long-term cultured NPCs lose their capacity to restrain the proliferation of pathogenic immune cells in vitro [147]. Therefore, it is imperative to obtain enough quantity of stem or progenitor cells within a short time before the quality of individual cell decreases. This presents a significant challenge for the technologies concerning iPSC-derived NSCs and directly induced NSCs.

Route of Administration

The mostly preferred routes for the delivery of MSCs or NSCs are the intravenous (i.v.) and intrathecal delivery routes since they can cross the blood-brain barrier (BBB) [148]. However, syngeneic naive NPCs injected subcutaneously and intravenously in EAE mice were low invasive in the CNS. Most of the injected NPCs were found in the liver, gut, spleen, lung, and kidney, which inevitably reduced the number of NPCs in secondary lymphoid organs and CNS [149, 150]. Focal injection of NSCs in the CNS is not practical in MS, where a multifocal, chronic, and spatially disseminated CNS damage accumulates over time. This would require multiple local injections to reach the multifocal lesions [151]. Intrathecal administration to lesions might be hindered by the limited capacity of grafted NSCs to migrate over long distances within the CNS parenchyma [152].

Delivery of NSCs directly into the cerebrospinal fluid (CSF) circulation by intracerebroventricular (i.c.v.) injection to specifically target the CNS in mice and rats has been tested [153]. Newborn rat NPCs, which were transplanted i.c.v. at the peak of disease in EAE, migrated exclusively into the inflamed white matter (but

not into adjacent gray matter regions) and subsequently differentiated into oligodendrocytes [154].

Intranasal (i.n.) delivery of NSCs is another noninvasive method of delivery. NSCs have shown to migrate into the CNS directly via the nasal route and result in functional recovery, and confer immunomodulation and remyelination in EAE in mice [155]. In mice, NSCs injected in the carotid artery promoted cell homing to the area of stroke lesion, and improved behavioral recovery [156]. Intracarotid delivery of NSC has not been reported in EAE. It has been shown that exogenous NSCs interact more closely with the infiltrating pathogenic immune cells rather than with those in the periphery. Therefore, suppression of inflammation in CNS by NSCs is likely to be more effective by targeted local delivery rather than their interaction at the periphery [155].

Therapeutic Mechanisms of Action of Transplanted NSCs: Studies in Animal Models of Demyelination

NSCs and NPCs have been shown to exert their beneficial effects through (a) immunomodulation, (b) cell replacement, (c) providing trophic support, and (d) stimulation of endogenous remyelination (Fig. 2) [157]. For the NSC therapy to be successful in MS, the cells need to be plastic enough to accommodate and survive in the nonpermissive inflammatory environment, highly migratory to reach multiple lesion sites in the CNS, and can differentiate into myelinating oligodendrocytes, through multiple mechanisms of action (Table 1).

Effect on T Cell Function

The immunomodulatory effects are mainly exerted by undifferentiated stem cells by releasing a milieu of neuroprotective molecules at the site of tissue lesion [158]. MSCs-NPCs have been shown to suppress T cell proliferation and to promote the expansion of FoxP3+ Treg cells in vitro [159]. NPCs induced from a human iPSC line were intraspinally transplanted into demyelinated mice due to viral infection and decreased the accumulation of CD4+ T cells in the CNS along with reduced demyelination at the site of injection which correlated with a transient increase in Treg cells in the peripheral lymphatics [145].

A recent study described long-lasting clinical recovery along with dampened neuroinflammation and remyelination after transplantation of NPCs derived from human ESCs, in a viral model of MS [160]. The human NPCs (hNPCs) used in that study were derived by a novel direct differentiation method (direct differentiation, DD-NPCs), and cells were selected for intraspinal transplantation based on a definitive transcriptomic signature. The same group then wanted to determine whether NPCs differentiated using conventional methods would be similarly effective in improving clinical outcome under neuroinflammatory demyelinating conditions. hNPCs were differentiated from a human iPSC line via the conventional embryoid body intermediate stage (EB-NPCs). Intraspinal transplantation of EB-NPCs into mice infected with the neurotropic JHMV resulted in decreased accumulation of CD4+ T cells in the central nervous system that was concomitant with reduced demyelination at the site of injection. Dampened neuroinflammation and remyelination was correlated with a transient increase in Treg cells concentrated within the peripheral lymphatics. However, compared to their earlier study, pathological improvements were modest and did not result in significant clinical recovery. It was concluded that the genetic signature of NPCs is critical to their effectiveness in this model. More importantly, there is a need for rigorous characterization and selection of therapeutically valuable NSC types derived from human iPSC for the treatment of MS [161].

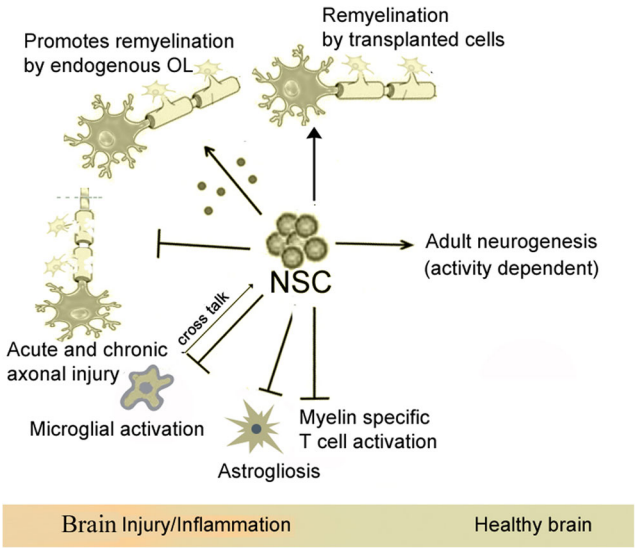

Fig. 2 Functions of adult NSCs in healthy and EAE (MS) brain. NSCs in the adult mammalian brain have been shown to give rise to rapidly dividing neural progenitor cells (NPCs) to produce neurons, astrocytes, and oligodendrocytes, and functionally contribute to (although modest) cognition and repair processes after injury. In EAE, NSCs have been shown to exert their beneficial effects through (a) immunomodulation, (b) cell replacement [153], (c) providing trophic support, and (d) stimulation of endogenous remyelination [166–168]. Transplanted NPCs can stimulate endogenous remyelination by inducing the proliferation and terminal differentiation of host OPCs, likely via CXCL12/CXCR4 autocrine signaling post inflammation [87]. NSCs inhibit MOG and MBP-specific CD4+ T cell activation, proliferation, and increased number of FOXP3+ Tregs cells [145, 150, 158, 160]. Intraspinally transplanted NPCs in postnatal mice can differentiate into mature oligodendrocytes and functionally incorporate throughout the demyelinated white matter tracts in JHMV-infected demyelination model [97]. NSCs can transform microglia from a harmful to a neuroprotective phenotype by significantly increasing the expression of molecules associated with a neuroprotective phenotype in adult mouse brain [119, 122–124]. Transplanted NSCs can indirectly suppress astrocyte gliosis in EAE

Table 1 Therapeutic mechanisms of action of transplanted NSCs in animal models of demyelination

Species/cell type source	Route	Type of EAE	Mechanism of action		Clinical outcomes	Ref.
			NSC to OPC differentiation	Immune modulation		
Mouse/adult SVZ NSCs	i.v.	Chronic EAE/mouse	Did not observe glial or neuronal differentiation	Inhibition of encephalitogenic T_H17 cell differentiation	Improvement in locomotor activity	[193]
Adult SVZ NSPCs (IL-10 producing)	i.c.v. and i.v.	Chronic EAE/mouse	Oligodendroglial and neuronal differentiation	Inhibition of peripheral and CNS-confined inflammation; induction of apoptosis of CNS-infiltrating T cells	Improvement of locomotor activity (EAE score)	[197]
Mouse/adult SVZ NSCs	i.c.v. and i.v.	Chronic EAE/mouse	Oligodendrocyte differentiation	Rescue of endogenous OPCs and modulation of neurotrophic and/or growth factors	Attenuation of EAE disease score and improvement of locomotor activity	[153]
Mouse/adult SVZ NSCs	i.v.	Relapsing EAE/mouse	Not reported/tested	Modulation of apoptosis of CNS-infiltrating T lymphocytes	Improvement of locomotor activity	[158, 163]
Rat/neonatal striatal derived NSC	i.c.v.	Acute EAE Rat	Not reported	Inhibition of MOG-specific lymphocyte proliferation	Improvement in locomotor activity and attenuation of clinical score	[154]
Mouse/humanEB-derived NSC	i.c.v.	Acute EAE viral-mouse		Remyelination was correlated with regulatory T cell induction		[145]
hESCs-derived NSC. Cells were selected based on a definitive transcriptomic signature	Intraspinal	Chronic EAE JHMV strain of mouse	NPC survival beyond 8 days posttransplantation not seen	Decreased accumulation of CD4+ T cells, reduced demyelination, and increase in CD4+FOXP3+ regulatory T cells (Tregs) in lymphatics	Significant neurological recovery	[160]
Mouse/MSC-NSC	s.c.	Acute EAE/mouse		(1) Suppressed the activation of myeloid DCs to APCs, (2) reduced the proliferation and activation of MOG-specific encephalitogenic T cells		[150]
Mouse/MSC-Sox2βGeo NPC	Inthrathecal	Acute EAE Mouse		(1) Exerted a neuroprotective effect via secretion of leukemia inhibitory factor (LIF), (2) was able to support the in vivo survival and differentiation of resident and oligodendrocytes, (3) attenuation of CNS inflammation reduced tissue injury	Amelioration of clinical and pathological features of the disease	[159]
Rat/fetal NSCs expressing IDO (rfNSCs-IDO)	i.v.	Acute EAE MOG-mouse	No glial cell differentiation	(1) Inhibition of T cell proliferation in peripheral lymph nodes, (2) increase in regulatory T cell numbers	Attenuated clinical scores (signs) and faster remission	[203]
Mice/bone marrow-derived NSCs overexpression IL-10, NT-3, and LINGO-1-Fc	i.v.	Acute EAE MOG-mouse	Glial cell differentiation seen. Blockade of further demyelination and the promotion of remyelination	Attenuation of CNS inflammation and promote an M2 phenotype in macrophages/microglia	Faster attenuation of clinical signs compared to control NSC	[209]
Rat/GDNF gene-modified NSCs (GDNF/NSCs)	i.c.v.	Acute EAE MOG-mouse	More glial and neuronal survival and differentiation compared to control NSC	Suppression of inflammation in the CNS	Attenuated the clinical signs and promotion of functional recovery	[192]

Trophic Support

MSC-NPCs are known to secrete trophic factors such as IGF-1, VEGF, HGF, and SDF-1 in vitro [159]. In EAE mice that were injected subcutaneously with NPCs prior to disease onset, the NPCs accumulated in the draining lymph nodes which hindered the activation of myeloid DCs to antigen-presenting cells (APCs) by a BMP-4-dependent mechanism, that reduced the proliferation and activation of encephalitogenic T cells [150]. Mac-3-, CD3-, and CD4-positive cells in the inflamed CNS were also diminished [162]. In chronic EAE, SVZ-derived syngenic NSCs promoted neuroprotection through secretion of immunomodulatory molecules and neurotrophic factors [163].

Intraventricular injections of newborn rat-derived NPCs into adult rats with acute EAE were shown to ameliorate the clinical severity and signs of EAE. Grafted NPCs migrated into the inflamed white matter and attenuated brain inflammation by inducing a reduction in perivascular infiltrates [164]. Syngeneic adult NSCs injected in the lateral ventricular were capable of long-distance migration into demyelinating areas inside an inflamed CNS in the EAE mice. Within these areas, OPCs of donor origin increased significantly and remyelinated axons actively [153].

Cell Replacement

Intraspinally transplanted NPCs in postnatal mice can differentiate into mature oligodendrocytes and functionally incorporate throughout the demyelinated white matter tracts in JHMV-infected demyelination model [97]. NPC transplantation did not alter the accumulation of T cells or macrophages within the CNS nor cytokine/chemokine gene expression in the CNS. Presumably, the enhanced remyelination was not dependent on bystander effects of grafted cells [98]. Transplantation of oligodendrocyte transcription factor 1 (Olig1) gene knockout NPCs (Olig1−/−) into JHMV-infected mice resulted in similar NSC survival, proliferation, and selective migration to areas of demyelination but exhibited poor remyelination. The majority of transplanted Olig1−/− NPCs differentiated into astrocyte lineage. These suggested that improved clinical symptoms might be associated with remyelination by the donor NSCs via formation of myelinating oligodendrocytes [165].

Stimulation of Endogenous Remyelination

Transplanted NPCs can stimulate endogenous remyelination by inducing the proliferation and terminal differentiation of host OPCs. NPCs that were transplanted into the lateral ventricles of cuprizone-fed mice were shown to exert a trophic effect on endogenous OPCs, and remyelination in the corpus callosum was performed exclusively by resident OPCs which failed to remyelinate in chronic MS [166]. Intrathecal injection of MSCs-NPCs at the onset of the chronic phase of disease increased the number of endogenous OPCs in EAE mice and accelerated remyelination [167]. These effects were manly exerted through the secretion of leukemia inhibitory factor (LIF) that promotes survival, differentiation, and remyelination capacity of endogenous OPCs and mature oligodendrocytes [168].

There are many differences in the inherent mechanisms between human NSCs and other mammal species-derived counterparts which should be worth of serious consideration in the translation of experimental research to the clinical setting. Intraspinal transplantation of human ES-NPCs in a viral model of MS resulted in dramatic reduction in neuroinflammation and sustained clinical recovery, although human NPCs were rejected within a relatively short period. Unlike the mouse NPCs, hNPCs had powerful immunomodulatory effects and induced an increased number of FOXP3+ Treg cells within the spinal cords [160]. There are more challenges to be tackled before NSC therapy in animal models can be safely and successfully translated to human therapy for MS [169].

The absence of CD95L in human NPCs during inflammation is unlikely to result in the massive T cell apoptosis reported in the mouse counterparts, whereas human NPCs have a higher capacity of generating oligodendrocyte cells in inflammatory conditions which are compatible with a therapeutic transplantation of NPCs for the treatment of MS [170].

Current Issues with NSC Transplantation: Effect of the Inflammatory Environment on NSC Survival and Differentiation

In MS and EAE, remyelination takes place within an inflammatory environment containing signals and chemicals that are intrinsically hostile to the survival and differentiation of oligodendrocyte [171]. In the adult brain, endogenous NSCs that are within the specialized germinal niches in the CNS are thought to provide support and maintenance to the endogenous OPCs. Direct physical contact and diffusible signals are the two major mechanisms that are thought to regulate the proliferation and differentiation of endogenous NSCs [172]. The in vivo differentiation of NSCs is highly dependent on the environmental cues within the CNS [173]. Identifying the mechanisms and signals responsible for blocking NSC differentiation in the CNS in MS warrants further investigation since manipulating these signals could promote oligodendrocyte production and remyelination, ultimately resulting in more effective CNS repair. Inflammation is permissive for the recruitment and migration of NSCs [74] while at the same time inhibitory to their proliferation and differentiation. The *Taiep* rat is a myelin mutant that shows many features of chronic demyelination in MS. The induction of acute inflammation in the nonremyelinating situation owing to a lack of

the stimuli required to activate OPCs to generate remyelinating oligodendrocytes results in remyelination [174]. An anti-inflammatory environment seems to be a prerequisite for the differentiation of NSCs into myelinating oligodendrocytes [175]. For example, the proinflammatory cytokine TNF- reduces the proliferative ability of NSCs and NPCs but induces their migration [173], whereas the anti-inflammatory cytokine IL-10 maintained NSCs in the adult brain of mice in undifferentiated yet highly proliferative state [176]. IFN-γ, an important cytokine for the clearance of CNS infections, inhibits the proliferation of NSPCs in inflammatory conditions through dephosphorylation of the tumor suppressor Retinoblastoma protein (pRb), which is dependent on activation of signal transducers and activators of transcription-1 (STAT1) signaling pathways [177]. From the foregoing discussion, it is apparent that inflammation is a double-edged sword as it could exert both detrimental and beneficial effects. Therefore, it is of great importance to determine the correct time of intervention, and design more refined therapies that aim at micromanipulating the inflammatory milieu in the CNS, and to offset the negative effects, and maximize the beneficial outcomes [178].

Differentiation arrest of transplanted and endogenous NPCs is the result of the persistent inflammatory environment prevailing in EAE and MS. Natural killer (NK) cells were in close proximity to NSCs in SVZ during the chronic phase of MS. NSCs produced interleukin-15 (IL-15) and sustain functionally competent NK cells which limited the neurorepair capacity of NSCs following brain inflammation [179]. At the acute phase of EAE, only a small fraction of NPCs injected in the lateral ventricle succeeded to differentiate, whereas at chronic phase, most of them followed a differentiation process [180].

NPCs display CNS pathotropism upon transplantation [181]. The clinical value of cell transplantation in a chronic, multifocal disease like MS will depend on the ability of transplanted cells to migrate to the multiple disease foci in the brain. The inflammatory process may attract targeted migration of transplanted cells into the inflammatory lesions. NSCs express CXCR4, the cognate receptor for SDF-1, and this inflammatory chemoattractant SDF-1/CXCR4 signaling is involved in the mobilization of NSCs toward the injury sites [182] and their differentiation into OPCs and mature oligodendrocytes upon focal transplantation into JHMV-infected mice with established demyelination [183].

The cellular densities and proliferative signals are significantly higher in MS SVZ as seen in postmortem MS brains [184]. Therefore, prolonged exposure of SVZ cells to repetitive inflammatory insults may not exhaust their proliferative potential. However, their migratory capability and oligodendrogenesis remain limited, implying that strategies aiming at promoting these phenomena need to be developed.

The progressive decline in the rate of proliferation of NSCs with aging raises the questions of whether the precursor cells eventually become unresponsive to cellular niche cues, or whether the cellular niche provides less positive stimuli for evoking proliferation or provides more negative cues [185]. Persistent CNS inflammation significantly impairs proliferation of stem/precursor cells in the SVZ of EAE mice by hindering their entry into the cell cycle by upregulation of cell cycle inhibitors, while these SVZ-resident cells return to normal kinetics once the inflammation subsides [186].

The continual and dual role of the neuroinflammatory response leaves it difficult to decipher upon a single modulatory strategy. To maximize the therapeutic effect of cell-based therapies, treatments must be specific to the injury and also be personalized for each patient [187].Therefore, developing a microenvironment conducive to the survival and proper differentiation of NSCs and in vitro induction prior to transplantation are of great importance for the application of NSCs to treat MS.

Genetically Modified NSCs

Genetic manipulation of NSCs holds great promise for improving the survivability of NSCs in vivo. Using various tools such as in vitro gene transfer, NSCs can be been manipulated for cell immortalization as well as control of proliferation. Genetically modified NSCs that overexpress prosurvival signaling molecules or paracrine factors, or critical glial cell lineage determining transcription factors, may enhance the therapeutic effects of NSC transplantation therapy. Trophic factors that are responsible for enhancing the survival, proliferation, and migration of transplanted NSCs provide neuroprotection, reduce astrogliosis, promote remyelination, and modulate inflammation. Specifically, NT-3, glial cell line-derived neurotrophic factor (GDNF), BDNF, IL-10, LIF, and Olig2 have been studied as potential candidates for genetic transduction to strengthen the efficacy and differentiation potential of NSCs into oligodendrocytes [188].

OPCs can be efficiently generated from human fetal NSCs by concurrent or sequential in vitro exposure to combinations of NT-3 and growth factors [189]. BM-NSCs transduced with NT-3 attenuated CNS inflammation and neurological deficits in active EAE significantly more than naive NSCs [190]. BM-NSCs exhibited efficient proliferation and differentiation into oligodendrocytes and neurons, and nominal differentiation into astrocytes, thus promoting remyelination and neuronal repopulation and reducing the degree of astrogliosis [188]. NT-3-induced BM-NSCs also secrete the anti-inflammatory cytokine IL-10, thus modulating a hostile host environment into a microenvironment supportive of remyelination [190].

GDNF gene-modified NSCs transplanted in the lateral ventricle of EAE rats significantly promoted functional recovery, profoundly suppressed brain inflammation, differentiated into

more neurons and oligodendrocytes, improved density of myelin, and reduced the clinical signs [191].

BDNF has been shown to play a key role in axon protection and disease attenuation during chronic EAE in mice [192]. BDNF was found to be elevated in the CSF of MS patients compared to control individuals, and CSF derived from both SPMS and PPMS patients significantly stimulated human embryonic-derived NPCs to differentiate into more oligodendrocytes in vitro [193]. Transplantation of human BDNF-NSCs significantly improved neurological motor function following traumatic brain injury (TBI) [194] and in middle cerebral artery occlusion model (MCAo) [195]. Human BM-NSCs and nanoparticle carriers encapsulated with BDNF and integrated into the biodegradable injectable 3D scaffolds increased secretion of LIF and chemokines by NSCs in the CNS and showed a sustained release of bioactive BDNF and enhanced their tissue repair [196].

Recent research demonstrates that adult mice CNS-derived NSCs engineered to secrete the anti-inflammatory cytokine IL-10 (IL-10-NSCs) exhibited enhanced peripheral immunosuppressive effects in EAE mice compared to naive NSCs [197]. IL-10-NSCs also promoted apoptosis of infiltrating T cells in the CNS through a Fas/FasL pathway and converted a hostile environment to a relatively more supportive of remyelination. Additionally, transplanted IL-10-NSCs differentiated primarily into oligodendrocytes at the expense of astrocyte generation. This was associated with significant attenuation of clinical signs and pathology in acute EAE compared to mice treated with control NSCs [198].

IGF-1 is critical for oligodendrocyte differentiation, survival, and myelination in neonatal and adult mice brain. IGF-1 produced by microglia and reactive astrocytes display protective effects on oligodendrocytes following cuprizone-induced toxic demyelination [199]. Transgenic mice that overexpressed IGF-1 demonstrated significantly less apoptosis of mature oligodendrocytes and exhibited rapid remyelination after cuprizone-induced demyelination [200, 201]. The IGF-1-overexpressing neonatal rats spinal cord-derived NSCs exhibited higher viability and efficiently differentiated into oligodendrocytes in a mouse spinal cord injury model [202]. The effects were shown to be mediated by extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathway.

NSCs normally express low levels of indoleamine 2,3-dioxygenase (IDO), a tryptophan-metabolizing enzyme which has potent immune suppressive activities. In an EAE animal model, systemic injections of NSCs expressing IDO resulted in significant local immune suppression in the cervical lymph nodes and CNS by recruiting regulatory T lymphocytes and reducing the number of activated T lymphocytes during the inflammation in the CNS which induced significantly fewer clinical symptoms and faster recovery [203].

Genetically altered NSCs that expressed the critical oligodendrocyte lineage transcription factor Olig2 promoted the functional recovery by contributing to remyelination and completely abrogating relapses when administered early after onset of EAE [204]. Most intraventricularly injected mice Olig2-NSCs differentiated into OPCs, in contrast to the control NSCs which largely remained undifferentiated [199]. Similarly, overexpression of Olig2 in mice SVZ progenitor cells increased the generation of OPCs which migrated and differentiated into mature oligodendrocytes after transplantation [205]. NSCs within the DG do not spontaneously differentiate into oligodendrocytes, and endogenous remyelination is limited after injury [206, 207]. However, retroviral mediated expression of the transcription factor Ascl1- into the DG of adult mice converted them into mature oligodendrocytes and enhanced their myelination in the DG in diphtheria-toxin (DT)-inducible, a genetic model for demyelination [207].

The chemokine (C-C motif) receptor 5 (CCR5) is a receptor for chemokines CCL3, CCL4, and CCL5, that are abundantly produced in the CNS-inflamed foci of MS/EAE. CCR5 overexpressing mouse BM-derived NSCs (CCR5-NSCs) were rapidly attracted toward inflamed foci in active EAE (in mice) in larger numbers and more effectively suppressed CNS inflammatory infiltration, thus reducing the extent of early myelin/neuron damage by creating a less hostile environment for host remyelinating cells [205].

NSCs could also be engineered to produce a "cocktail" of potential therapeutic molecules effectively targeting the major mechanisms underlying the chronicity of EAE and MS, such as persistent inflammation, deficiency of trophic support for differentiation, and accumulation of neuroregeneration inhibitors. Soluble LINGO-1 protein (LINGO-1-Fc), an antagonist of LINGO-1, is a key part of the common receptor complex which blocks the harmful effect of neuroregeneration inhibitors on OPCs/oligodendrocytes and attenuates myelin inhibition [208]. At the chronic stage of EAE, NSCs engineered to produce IL-10 (for immunosuppression), NT-3 (for neurotrophy), and LINGO-1-Fc (for inhibition of negative effects) migrated into the inflamed foci and induced M2 macrophages/microglia in CNS, thus reducing astrogliosis and promoting endogenous oligodendrocyte/neuron differentiation which represents a novel and potentially effective therapy for the chronic stage of MS [209].

Immortalized human NSC cell lines can be generated by a retroviral vector encoded with a v-myc oncogene. These immortalized NSCs exhibited potent migration capability and differentiation potential into neurons and glial cells in animal models of human neurological disorders. Multipotent neural cell lines can engraft and participate in the development of mouse cerebellum [210]. The continuously multiplying cell may exist as a limitless supply of neurons and oligodendrocytes for the treatment for MS [211]. Although Fas-deficient NPCs had significantly higher survival and increased differentiation capabilities compared to wild-type NPCs

in vitro, this did not translate to better terminal differentiation and posttransplantation survival in vivo. The environmental factors in the CNS prevented the differentiation of grafted NPCs, regardless of their inherent differentiation capacities ex vivo [212].

Genetically engineered NSC can boost and influence multiple gene networks and interacts with endogenous neural and immune cells to improve cognitive and motor behavior. Expression of specific, transcription factors, or ligands or receptors in NSC can induce relatively more significant changes in synaptic plasticity and mitochondrial and lysosomal function and affect both innate and adaptive immunity resulting in better functional recovery. Alternatively, they can be generated as more fate restrictive, to direct them to generate more glial cells for remyelination.

Clinical Research on NSC-Based Cell Therapies

Safety is the primary concern of stem cell therapies; clinical researches on NSCs in MS have not been reported to date. In an early study, 15 patients with amyotrophic lateral sclerosis (ALS) receiving an intraspinal transplantation of escalating doses of NSCs safely tolerated the cells at high doses [213]. A recent pilot study investigated the safety and tolerability of autologous MSC-NPCs treatment for MS. Six patients with progressive MS who were refractory to conventional treatments were treated with intrathecal injections of MSC-NPCs, and there were no serious adverse events in the following 7 years and some patients showed a measurable clinical improvement [214]. The same authors reported a phase 1 safety trial involving 20 MS patients with established disability, in which MSC-NPs administered intrathecally in three doses of up to 10 million cells per injection, spaced three months apart, resulted in improved Expanded Disability Status Scale (EDSS), improved 9-Hole Peg Test (9-HPT), and better bladder function clinically (reported as abstract and oral presentation at the 68th Annual Meeting of the American Academy of Neurology). A phase I, open-label, single-site, safety study of human spinal cord-derived neural stem cell (supplied by Stem Cell Incorporation) transplantation for the treatment of chronic spinal cord injury has been initiated in four spinal cord injury (SCI) patients in 2016, which was well tolerated. Data is still being collected. A phase I safety study was conducted by Dr. David Rowitch's group for testing human fetal CNS-derived neural stem cell transplantation in four Pelizaeus-Merzbacher disease (PMD) subjects. The cells were fairly tolerated with no serious or fatal outcomes. A fraction of the patients had a modest but clear gain in motor functions, which are not seen for such a progressive and severe neurodegenerative disease [215]. Based on increasing evidence demonstrating the robust regenerative potential of human NSCs, this mode of cell therapy could provide a feasible clinical intervention in stopping neurodegeneration. In theory, a combination therapy with existing immunomodulatory therapies may be beneficial, i.e., simultaneously replacing cells, regulating autoimmunity, and promoting regeneration in MS patients.

Conclusions

The present review delineates several aspects of the MS pathology, endogenous remyelination, and results of NSC transplantation in animal models that must be taken into consideration in the development of an NSC-based cell therapy for MS. We briefly summarized the current understanding of MS pathogenesis, namely the different types of pathological lesions in the CNS, immune cell-mediated inflammatory demyelination, apoptosis of oligodendrocytes, axonal degeneration, and oxidative stress. The current consensus regarding an effective therapeutic regimen was that the treatment should contain a combination of anti-inflammatory, regenerative, and neuroprotective strategies. The success of NSC transplantation primarily depends on the cell fate precommitment of transplanted NSCs into OPCs, while at the same time the endogenous differentiation of OPCs needs to be boosted in chronic stages of the disease. Preclinical data suggests that NSCs and NPCs may be competent in simultaneously exerting an immunomodulatory action as well as activation of the endogenous NSC pool. Modulation of microglial function in the CNS is an important target for NSCs. However, the activity of microglia in a different stage of MS is different; therefore, optimum timing of interventions needs to be carefully explored. The extent of cell replacement is currently not clear and needs further exploration. However, several complex issues need to be addressed. First, large-scale generation of NSCs or NPCs from human iPSCs or by direct conversion of somatic cells into iNSCs must be developed. There is also a need for rigorous characterization and selection of therapeutically valuable NSC types derived from human iPSCs. Lastly, the ideal route and time of NSC injection are of great importance since the fate of transplanted cells, therapeutic mechanisms, and efficacy in vivo are critically dependent on these factors. Genetically modified NSCs expressing trophic or survival factors could improve the microenvironments, enhancing the survival and appropriate differentiation of NSCs. The behavior and efficacy of exogenous NSCs in different types of animal models need comprehensive analysis to deduce the real features of NSCs before translation into clinical trials. Assisting the endogenous stem cells to overcome the obstacles of proliferation, migration, and differentiation in the lesions is another interesting approach, and humanized mice models are needed to simulate the scenarios.

Acknowledgements We thank the funding support from the National Natural Science Foundation of China (81601373), Hubei Provincial Natural Science Foundation of China (2016CFB407), Bureau of Xiangyang City Science and Technology projects (No.[2014]6-7), Project for Discipline Groups Construction of Food New-type Industrialization of Hubei University of Arts and Science, National Institutes of Health (R01HD087566), National Multiple Sclerosis Society, and Shriners Hospitals for Children.

Authors' Contributions All authors researched the data for the article, provided substantial contributions to discussions of the content, and wrote the article.

Compliance with Ethical Standards

Competing Interests The authors declare that they have no competing interests.

References

1. Lassmann H (2013) Pathology and disease mechanisms in different stages of multiple sclerosis. J Neurol Sci 333(1–2):1–4. doi:10.1016/j.jns.2013.05.010
2. Kishore A, Kanaujia A, Nag S, Rostami AM, Kenyon LC, Shindler KS, Das Sarma J (2013) Different mechanisms of inflammation induced in virus and autoimmune-mediated models of multiple sclerosis in C57BL6 mice. Biomed Res Int 2013: 589048. doi:10.1155/2013/589048
3. Confavreux C, Vukusic S, Moreau T, Adeleine P (2000) Relapses and progression of disability in multiple sclerosis. N Engl J Med 343(20):1430–1438. doi:10.1056/NEJM200011163432001
4. Miller DH, Leary SM (2007) Primary-progressive multiple sclerosis. Lancet Neurol 6(10):903–912. doi:10.1016/S1474-4422(07)70243-0
5. Fugger L, Friese MA, Bell JI (2009) From genes to function: the next challenge to understanding multiple sclerosis. Nat Rev Immunol 9(6):408–417. doi:10.1038/nri2554
6. Perga S, Montarolo F, Martire S, Berchialla P, Malucchi S, Bertolotto A (2015) Anti-inflammatory genes associated with multiple sclerosis: a gene expression study. J Neuroimmunol 279:75–78. doi:10.1016/j.jneuroim.2015.01.004
7. Kohm AP, Carpentier PA, Anger HA, Miller SD (2002) Cutting edge: CD4+CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. J Immunol 169(9):4712–4716
8. Eliseeva DD, Zavalishin IA, Karaulov AV, Bykovskaia SN (2012) [The role of regulatory T cells in the development of autoimmune process in multiple sclerosis]. Vestnik Rossiiskoi akademii meditsinskikh nauk / Rossiiskaia akademiia meditsinskikh nauk (3):68–74
9. Peelen E, Damoiseaux J, Smolders J, Knippenberg S, Menheere P, Tervaert JW, Hupperts R, Thewissen M (2011) Th17 expansion in MS patients is counterbalanced by an expanded CD39+ regulatory T cell population during remission but not during relapse. J Neuroimmunol 240-241:97–103. doi:10.1016/j.jneuroim.2011.09.013
10. Lifshitz GV, Zhdanov DD, Lokhonina AV, Eliseeva DD, Lyssuck EY, Zavalishin IA, Bykovskaia SN (2016) Ex vivo expanded regulatory T cells CD4+CD25+FoxP3+CD127Low develop strong immunosuppressive activity in patients with remitting-relapsing multiple sclerosis. Autoimmunity 49(6):388–396. doi:10.1080/08916934.2016.1199020
11. Zozulya AL, Wiendl H (2008) The role of regulatory T cells in multiple sclerosis. Nat Clin Pract Neurol 4(7):384–398. doi:10.1038/ncpneuro0832
12. Koch MW, Metz LM, Agrawal SM, Yong VW (2013) Environmental factors and their regulation of immunity in multiple sclerosis. J Neurol Sci 324(1–2):10–16. doi:10.1016/j.jns.2012.10.021
13. Munger KL, Zhang SM, O'Reilly E, Hernan MA, Olek MJ, Willett WC, Ascherio A (2004) Vitamin D intake and incidence of multiple sclerosis. Neurology 62(1):60–65
14. Haider L (2015) Inflammation, iron, energy failure, and oxidative stress in the pathogenesis of multiple sclerosis. Oxidative Med Cell Longev 2015:725370. doi:10.1155/2015/725370
15. Odoardi F, Sie C, Streyl K, Ulaganathan VK, Schlager C, Lodygin D, Heckelsmiller K, Nietfeld W et al (2012) T cells become licensed in the lung to enter the central nervous system. Nature 488(7413):675–679. doi:10.1038/nature11337
16. Correale J, Farez MF (2015) Smoking worsens multiple sclerosis prognosis: two different pathways are involved. J Neuroimmunol 281:23–34. doi:10.1016/j.jneuroim.2015.03.006
17. De Jager PL, Simon KC, Munger KL, Rioux JD, Hafler DA, Ascherio A (2008) Integrating risk factors: HLA-DRB1*1501 and Epstein-Barr virus in multiple sclerosis. Neurology 70(13 Pt 2):1113–1118. doi:10.1212/01.wnl.0000294325.63006.f8
18. Cepok S, Zhou D, Srivastava R, Nessler S, Stei S, Bussow K, Sommer N, Hemmer B (2005) Identification of Epstein-Barr virus proteins as putative targets of the immune response in multiple sclerosis. J Clin Invest 115(5):1352–1360. doi:10.1172/JCI23661
19. Virtanen JO, Farkkila M, Multanen J, Uotila L, Jaaskelainen AJ, Vaheri A, Koskiniemi M (2007) Evidence for human herpesvirus 6 variant A antibodies in multiple sclerosis: diagnostic and therapeutic implications. J Neurovirol 13(4):347–352. doi:10.1080/13550280701381332
20. Pohl D (2009) Epstein-Barr virus and multiple sclerosis. J Neurol Sci 286(1–2):62–64. doi:10.1016/j.jns.2009.03.028
21. Pender MP, Csurhes PA, Smith C, Beagley L, Hooper KD, Raj M, Coulthard A, Burrows SR et al (2014) Epstein-Barr virus-specific adoptive immunotherapy for progressive multiple sclerosis. Mult Scler 20(11):1541–1544. doi:10.1177/1352458514521888
22. Tsunoda I, Fujinami RS (2002) Inside-out versus outside-in models for virus induced demyelination: axonal damage triggering demyelination. Springer Semin Immunopathol 24(2):105–125. doi:10.1007/s00281-002-0105-z
23. Amor S, Puentes F, Baker D, van der Valk P (2010) Inflammation in neurodegenerative diseases. Immunology 129(2):154–169. doi:10.1111/j.1365-2567.2009.03225.x
24. Sato F, Martinez NE, Stewart EC, Omura S, Alexander JS, Tsunoda I (2015) "Microglial nodules" and "newly forming lesions" may be a Janus face of early MS lesions; implications from virus-induced demyelination, the inside-out model. BMC Neurol 15:219. doi:10.1186/s12883-015-0478-y
25. Hagemeier K, Bruck W, Kuhlmann T (2012) Multiple sclerosis—remyelination failure as a cause of disease progression. Histol Histopathol 27(3):277–287
26. Stohlman SA, Hinton DR (2001) Viral induced demyelination. Brain Pathol 11(1):92–106
27. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L (1998) Axonal transection in the lesions of multiple sclerosis. N Engl J Med 338(5):278–285. doi:10.1056/NEJM199801293380502
28. Joshi DC, Zhang CL, Lin TM, Gusain A, Harris MG, Tree E, Yin Y, Wu C et al (2015) Deletion of mitochondrial anchoring protects dysmyelinating shiverer: implications for progressive. Ms 35(13): 5293–5306. doi:10.1523/jneurosci.3859-14.2015

29. Trapp BD, Nave KA (2008) Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci 31:247–269. doi:10.1146/annurev.neuro.30.051606.094313
30. Birgbauer E, Rao TS, Webb M (2004) Lysolecithin induces demyelination in vitro in a cerebellar slice culture system. J Neurosci Res 78(2):157–166. doi:10.1002/jnr.20248
31. Andrews H, White K, Thomson C, Edgar J, Bates D, Griffiths I, Turnbull D, Nichols P (2006) Increased axonal mitochondrial activity as an adaptation to myelin deficiency in the Shiverer mouse. J Neurosci Res 83(8):1533–1539. doi:10.1002/jnr.20842
32. Trebst C, Heine S, Lienenklaus S, Lindner M, Baumgartner W, Weiss S, Stangel M (2007) Lack of interferon-beta leads to accelerated remyelination in a toxic model of central nervous system demyelination. Acta Neuropathol 114(6):587–596. doi:10.1007/s00401-007-0300-z
33. Witte ME, Bo L, Rodenburg RJ, Belien JA, Musters R, Hazes T, Wintjes LT, Smeitink JA et al (2009) Enhanced number and activity of mitochondria in multiple sclerosis lesions. J Pathol 219(2):193–204. doi:10.1002/path.2582
34. Popescu BF, Lucchinetti CF (2012) Pathology of demyelinating diseases. Annu Rev Pathol 7:185–217. doi:10.1146/annurev-pathol-011811-132443
35. Grade S, Bernardino L, Malva JO (2013) Oligodendrogenesis from neural stem cells: perspectives for remyelinating strategies. Int J Dev Neurosci 31(7):692–700. doi:10.1016/j.ijdevneu.2013.01.004
36. Prineas JW, Barnard RO, Kwon EE, Sharer LR, Cho ES (1993) Multiple sclerosis: remyelination of nascent lesions. Ann Neurol 33(2):137–151. doi:10.1002/ana.410330203
37. Fancy SP, Zhao C, Franklin RJ (2004) Increased expression of Nkx2.2 and Olig2 identifies reactive oligodendrocyte progenitor cells responding to demyelination in the adult CNS. Mol Cell Neurosci 27(3):247–254. doi:10.1016/j.mcn.2004.06.015
38. Wilson HC, Scolding NJ, Raine CS (2006) Co-expression of PDGF alpha receptor and NG2 by oligodendrocyte precursors in human CNS and multiple sclerosis lesions. J Neuroimmunol 176(1–2):162–173. doi:10.1016/j.jneuroim.2006.04.014
39. Cai J, Qi Y, Hu X, Tan M, Liu Z, Zhang J, Li Q, Sander M et al (2005) Generation of oligodendrocyte precursor cells from mouse dorsal spinal cord independent of Nkx6 regulation and Shh signaling. Neuron 45(1):41–53. doi:10.1016/j.neuron.2004.12.028
40. Mecha M, Feliu A, Carrillo-Salinas FJ, Mestre L, Guaza C (2013) Mobilization of progenitors in the subventricular zone to undergo oligodendrogenesis in the Theiler's virus model of multiple sclerosis: implications for remyelination at lesions sites. Exp Neurol 250:348–352. doi:10.1016/j.expneurol.2013.10.011
41. Xing YL, Roth PT, Stratton JA, Chuang BH, Danne J, Ellis SL, Ng SW, Kilpatrick TJ et al (2014) Adult neural precursor cells from the subventricular zone contribute significantly to oligodendrocyte regeneration and remyelination. J Neurosci 34(42):14128–14146. doi:10.1523/jneurosci.3491-13.2014
42. Staugaitis SM, Chang A, Trapp BD (2012) Cortical pathology in multiple sclerosis: experimental approaches to studies on the mechanisms of demyelination and remyelination. Acta Neurol Scand Suppl 195:97–102. doi:10.1111/ane.12041
43. Dulamea AO (2017) Role of oligodendrocyte dysfunction in demyelination, remyelination and neurodegeneration in multiple sclerosis. Adv Exp Med Biol 958:91–127. doi:10.1007/978-3-319-47861-6_7
44. Mason JL, Toews A, Hostettler JD, Morell P, Suzuki K, Goldman JE, Matsushima GK (2004) Oligodendrocytes and progenitors become progressively depleted within chronically demyelinated lesions. Am J Pathol 164(5):1673–1682. doi:10.1016/S0002-9440(10)63726-1
45. Zawadzka M, Rivers LE, Fancy SP, Zhao C, Tripathi R, Jamen F, Young K, Goncharevich A et al (2010) CNS-resident glial progenitor/stem cells produce Schwann cells as well as oligodendrocytes during repair of CNS demyelination. Cell Stem Cell 6(6):578–590. doi:10.1016/j.stem.2010.04.002
46. Chang A, Nishiyama A, Peterson J, Prineas J, Trapp BD (2000) NG2-positive oligodendrocyte progenitor cells in adult human brain and multiple sclerosis lesions. J Neurosci 20(17):6404–6412
47. Armstrong RC, Le TQ, Flint NC, Vana AC, Zhou YX (2006) Endogenous cell repair of chronic demyelination. J Neuropathol Exp Neurol 65(3):245–256. doi:10.1097/01.jnen.0000205142.08716.7e
48. Reynolds R, Dawson M, Papadopoulos D, Polito A, Di Bello IC, Pham-Dinh D, Levine J (2002) The response of NG2-expressing oligodendrocyte progenitors to demyelination in MOG-EAE and MS. J Neurocytol 31(6–7):523–536
49. Lassmann H, Bruck W, Lucchinetti CF (2007) The immunopathology of multiple sclerosis: an overview. Brain Pathol 17(2):210–218. doi:10.1111/j.1750-3639.2007.00064.x
50. Hoftberger R, Aboul-Enein F, Brueck W, Lucchinetti C, Rodriguez M, Schmidbauer M, Jellinger K, Lassmann H (2004) Expression of major histocompatibility complex class I molecules on the different cell types in multiple sclerosis lesions. Brain Pathol 14(1):43–50
51. Saxena A, Bauer J, Scheikl T, Zappulla J, Audebert M, Desbois S, Waisman A, Lassmann H et al (2008) Cutting edge: multiple sclerosis-like lesions induced by effector CD8 T cells recognizing a sequestered antigen on oligodendrocytes. J Immunol 181(3):1617–1621
52. Barnett MH, Prineas JW (2004) Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann Neurol 55(4):458–468. doi:10.1002/ana.20016
53. McTigue DM, Tripathi RB (2008) The life, death, and replacement of oligodendrocytes in the adult CNS. J Neurochem 107(1):1–19. doi:10.1111/j.1471-4159.2008.05570.x
54. Thorburne SK, Juurlink BH (1996) Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. J Neurochem 67(3):1014–1022
55. Tavazzi E, Rovaris M, La Mantia L (2014) Drug therapy for multiple sclerosis. CMAJ 186(11):833–840. doi:10.1503/cmaj.130727
56. Weiner HL (2009) The challenge of multiple sclerosis: how do we cure a chronic heterogeneous disease? Ann Neurol 65(3):239–248. doi:10.1002/ana.21640
57. Fitzner D, Simons M (2010) Chronic progressive multiple sclerosis—pathogenesis of neurodegeneration and therapeutic strategies. Curr Neuropharmacol 8(3):305–315. doi:10.2174/157015910792246218
58. Steinman L, Zamvil SS (2006) How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. Ann Neurol 60(1):12–21. doi:10.1002/ana.20913
59. Stys PK, Zamponi GW, van Minnen J, Geurts JJ (2012) Will the real multiple sclerosis please stand up? Nat Rev Neurosci 13(7):507–514. doi:10.1038/nrn3275
60. Sato F, Tanaka H, Hasanovic F, Tsunoda I (2011) Theiler's virus infection: pathophysiology of demyelination and neurodegeneration. Pathophysiology 18(1):31–41. doi:10.1016/j.pathophys.2010.04.011
61. Oikonen M, Laaksonen M, Aalto V, Ilonen J, Salonen R, Eralinna JP, Panelius M, Salmi A (2011) Temporal relationship between environmental influenza A and Epstein-Barr viral infections and high multiple sclerosis relapse occurrence. Mult Scler 17(6):672–680. doi:10.1177/1352458510394397
62. Procaccini C, De Rosa V, Pucino V, Formisano L, Matarese G (2015) Animal models of multiple sclerosis. Eur J Pharmacol 759:182–191. doi:10.1016/j.ejphar.2015.03.042

63. von Herrath MG, Fujinami RS, Whitton JL (2003) Microorganisms and autoimmunity: making the barren field fertile? Nat Rev Microbiol 1(2):151–157. doi:10.1038/nrmicro754
64. Keough MB, Jensen SK, Yong VW (2015) Experimental demyelination and remyelination of murine spinal cord by focal injection of lysolecithin. J Vis Exp 97. doi:10.3791/52679
65. Baxi EG, DeBruin J (2015) Transfer of myelin-reactive th17 cells impairs endogenous remyelination in the central nervous system of cuprizone-fed mice. J Neurosci 35(22):8626–8639. doi:10.1523/jneurosci.3817-14.2015
66. Cristofanilli M, Rosenthal H, Cymring B, Gratch D, Pagano B, Xie B, Sadiq SA (2014) Progressive multiple sclerosis cerebrospinal fluid induces inflammatory demyelination, axonal loss, and astrogliosis in mice. Exp Neurol 261:620–632. doi:10.1016/j.expneurol.2014.07.020
67. Alvarez-Buylla A, Lim DA (2004) For the long run: maintaining germinal niches in the adult brain. Neuron 41(5):683–686
68. Nunes MC, Roy NS, Keyoung HM, Goodman RR, McKhann G 2nd, Jiang L, Kang J, Nedergaard M et al (2003) Identification and isolation of multipotential neural progenitor cells from the subcortical white matter of the adult human brain. Nat Med 9(4):439–447. doi:10.1038/nm837
69. Luo Y, Coskun V, Liang A, Yu J, Cheng L, Ge W, Shi Z, Zhang K et al (2015) Single-cell transcriptome analyses reveal signals to activate dormant neural stem cells. Cell 161(5):1175–1186. doi:10.1016/j.cell.2015.04.001
70. Andressen C (2013) Neural stem cells: from neurobiology to clinical applications. Curr Pharm Biotechnol 14(1):20–28
71. Ming GL, Song H (2011) Adult neurogenesis in the mammalian brain: significant answers and significant questions. Neuron 70(4):687–702. doi:10.1016/j.neuron.2011.05.001
72. Nait-Oumesmar B, Decker L, Lachapelle F, Avellana-Adalid V, Bachelin C, Baron-Van Evercooren A (1999) Progenitor cells of the adult mouse subventricular zone proliferate, migrate and differentiate into oligodendrocytes after demyelination. Eur J Neurosci 11(12):4357–4366
73. Jablonska A, Kozlowska H, Markiewicz I, Domanska-Janik K, Lukomska B (2010) Transplantation of neural stem cells derived from human cord blood to the brain of adult and neonatal rats. Acta Neurobiol Exp 70(4):337–350
74. Picard-Riera N, Decker L, Delarasse C, Goude K, Nait-Oumesmar B, Liblau R, Pham-Dinh D, Baron-Van Evercooren A (2002) Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. Proc Natl Acad Sci U S A 99(20):13211–13216. doi:10.1073/pnas.192314199
75. Jessberger S, Toni N, Clemenson GD Jr, Ray J, Gage FH (2008) Directed differentiation of hippocampal stem/progenitor cells in the adult brain. Nat Neurosci 11(8):888–893. doi:10.1038/nn.2148
76. Tramontin AD, Garcia-Verdugo JM, Lim DA, Alvarez-Buylla A (2003) Postnatal development of radial glia and the ventricular zone (VZ): a continuum of the neural stem cell compartment. Cereb Cortex 13(6):580–587
77. Menn B, Garcia-Verdugo JM, Yaschine C, Gonzalez-Perez O, Rowitch D, Alvarez-Buylla A (2006) Origin of oligodendrocytes in the subventricular zone of the adult brain. J Neurosci 26(30):7907–7918. doi:10.1523/JNEUROSCI.1299-06.2006
78. Capilla-Gonzalez V, Herranz-Perez V, Garcia-Verdugo JM (2015) The aged brain: genesis and fate of residual progenitor cells in the subventricular zone. Front Cell Neurosci 9:365. doi:10.3389/fncel.2015.00365
79. Conover JC, Shook BA (2011) Aging of the subventricular zone neural stem cell niche. Aging Dis 2(1):49–63
80. Capilla-Gonzalez V, Cebrian-Silla A, Guerrero-Cazares H, Garcia-Verdugo JM, Quinones-Hinojosa A (2014) Age-related changes in astrocytic and ependymal cells of the subventricular zone. Glia 62(5):790–803. doi:10.1002/glia.22642
81. Encinas JM, Michurina TV, Peunova N, Park JH, Tordo J, Peterson DA, Fishell G, Koulakov A et al (2011) Division-coupled astrocytic differentiation and age-related depletion of neural stem cells in the adult hippocampus. Cell Stem Cell 8(5):566–579. doi:10.1016/j.stem.2011.03.010
82. Samanta J, Grund EM, Silva HM, Lafaille JJ, Fishell G, Salzer JL (2015) Inhibition of Gli1 mobilizes endogenous neural stem cells for remyelination. Nature 526(7573):448–452. doi:10.1038/nature14957
83. Klingener M, Chavali M, Singh J, McMillan N, Coomes A, Dempsey PJ, Chen EI, Aguirre A (2014) N-cadherin promotes recruitment and migration of neural progenitor cells from the SVZ neural stem cell niche into demyelinated lesions. J Neurosci 34(29):9590–9606. doi:10.1523/JNEUROSCI.3699-13.2014
84. Brousse B, Magalon K, Durbec P, Cayre M (2015) Region and dynamic specificities of adult neural stem cells and oligodendrocyte precursors in myelin regeneration in the mouse brain. Biol Open 4(8):980–992. doi:10.1242/bio.012773
85. Calzolari F, Michel J, Baumgart EV, Theis F, Gotz M, Ninkovic J (2015) Fast clonal expansion and limited neural stem cell self-renewal in the adult subependymal zone. Nat Neurosci 18(4):490–492. doi:10.1038/nn.3963
86. Lee Y, Oh SB, Park HR, Kim HS, Kim MS, Lee J (2013) Selective impairment on the proliferation of neural progenitor cells by oxidative phosphorylation disruption. Neurosci Lett 535:134–139. doi:10.1016/j.neulet.2012.12.050
87. Zilkha-Falb R, Kaushansky N, Kawakami N, Ben-Nun A (2016) Post-CNS-inflammation expression of CXCL12 promotes the endogenous myelin/neuronal repair capacity following spontaneous recovery from multiple sclerosis-like disease. J Neuroinflammation 13:7. doi:10.1186/s12974-015-0468-4
88. Kaneko N, Kako E, Sawamoto K (2011) Prospects and limitations of using endogenous neural stem cells for brain regeneration. Genes 2(1):107–130. doi:10.3390/genes2010107
89. Shirazi HA, Rasouli J, Ciric B, Rostami A, Zhang GX (2015) 1, 25-Dihydroxyvitamin D3 enhances neural stem cell proliferation and oligodendrocyte differentiation. Exp Mol Pathol 98(2):240–245. doi:10.1016/j.yexmp.2015.02.004
90. Goudarzvand M, Javan M, Mirnajafi-Zadeh J, Mozafari S, Tiraihi T (2010) Vitamins E and D3 attenuate demyelination and potentiate remyelination processes of hippocampal formation of rats following local injection of ethidium bromide. Cell Mol Neurobiol 30(2):289–299. doi:10.1007/s10571-009-9451-x
91. Penna G, Roncari A, Amuchastegui S, Daniel KC, Berti E, Colonna M, Adorini L (2005) Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+ Foxp3+ regulatory T cells by 1,25-dihydroxyvitamin D3. Blood 106(10):3490–3497. doi:10.1182/blood-2005-05-2044
92. Toghianifar N, Ashtari F, Zarkesh-Esfahani SH, Mansourian M (2015) Effect of high dose vitamin D intake on interleukin-17 levels in multiple sclerosis: a randomized, double-blind, placebo-controlled clinical trial. J Neuroimmunol 285:125–128. doi:10.1016/j.jneuroim.2015.05.022
93. Cantarella C, Cayre M, Magalon K, Durbec P (2008) Intranasal HB-EGF administration favors adult SVZ cell mobilization to demyelinated lesions in mouse corpus callosum. Dev Neurobiol 68(2):223–236. doi:10.1002/dneu.20588
94. Holley JE, Gveric D, Newcombe J, Cuzner ML, Gutowski NJ (2003) Astrocyte characterization in the multiple sclerosis glial scar. Neuropathol Appl Neurobiol 29(5):434–444
95. Amir-Levy Y, Mausner-Fainberg K, Karni A (2014) Treatment with anti-EGF ab ameliorates experimental autoimmune

encephalomyelitis via induction of neurogenesis and oligodendrogenesis. Mult Scler Int 2014:926134. doi:10.1155/2014/926134
96. Cate HS, Sabo JK, Merlo D, Kemper D, Aumann TD, Robinson J, Merson TD, Emery B et al (2010) Modulation of bone morphogenic protein signalling alters numbers of astrocytes and oligodendroglia in the subventricular zone during cuprizone-induced demyelination. J Neurochem 115(1):11–22. doi:10.1111/j.1471-4159.2010.06660.x
97. Totoiu MO, Nistor GI, Lane TE, Keirstead HS (2004) Remyelination, axonal sparing, and locomotor recovery following transplantation of glial-committed progenitor cells into the MHV model of multiple sclerosis. Exp Neurol 187(2):254–265. doi:10.1016/j.expneurol.2004.01.028
98. Hardison JL, Nistor G, Gonzalez R, Keirstead HS, Lane TE (2006) Transplantation of glial-committed progenitor cells into a viral model of multiple sclerosis induces remyelination in the absence of an attenuated inflammatory response. Exp Neurol 197(2):420–429. doi:10.1016/j.expneurol.2005.10.016
99. Blanc CA, Grist JJ, Rosen H, Sears-Kraxberger I, Steward O, Lane TE (2015) Sphingosine-1-phosphate receptor antagonism enhances proliferation and migration of engrafted neural progenitor cells in a model of viral-induced demyelination. Am J Pathol 185(10):2819–2832. doi:10.1016/j.ajpath.2015.06.009
100. Guan Y, Jiang Z, Ciric B, Rostami AM, Zhang GX (2008) Upregulation of chemokine receptor expression by IL-10/IL-4 in adult neural stem cells. Exp Mol Pathol 85(3):232–236. doi:10.1016/j.yexmp.2008.07.003
101. Harry GJ, Kraft AD (2012) Microglia in the developing brain: a potential target with lifetime effects. Neurotoxicology 33(2):191–206. doi:10.1016/j.neuro.2012.01.012
102. Suzumura A (2013) Microglia in pathophysiology of neuroimmunological disorders. Nihon rinsho Jpn J Clin Med 71(5):801–806
103. van Horssen J, Singh S, van der Pol S, Kipp M, Lim JL, Peferoen L, Gerritsen W, Kooi EJ et al (2012) Clusters of activated microglia in normal-appearing white matter show signs of innate immune activation. J Neuroinflammation 9:156. doi:10.1186/1742-2094-9-156
104. Perry VH, Nicoll JA, Holmes C (2010) Microglia in neurodegenerative disease. Nat Rev Neurol 6(4):193–201. doi:10.1038/nrneurol.2010.17
105. Al-Shamsi M, Shahin A, Ibrahim MF, Tareq S, Souid AK, Mensah-Brown EP (2015) Bioenergetics of the spinal cord in experimental autoimmune encephalitis of rats. BMC Neurosci 16:37. doi:10.1186/s12868-015-0175-1
106. Lampron A, Larochelle A, Laflamme N, Prefontaine P, Plante MM, Sanchez MG, Yong VW, Stys PK et al (2015) Inefficient clearance of myelin debris by microglia impairs remyelinating processes. J Exp Med 212(4):481–495. doi:10.1084/jem.20141656
107. Ramesh G, Benge S, Pahar B, Philipp MT (2012) A possible role for inflammation in mediating apoptosis of oligodendrocytes as induced by the Lyme disease spirochete Borrelia burgdorferi. J Neuroinflammation 9:72. doi:10.1186/1742-2094-9-72
108. Peferoen L, Kipp M, van der Valk P, van Noort JM, Amor S (2014) Oligodendrocyte-microglia cross-talk in the central nervous system. Immunology 141(3):302–313. doi:10.1111/imm.12163
109. Prineas JW, Kwon EE, Cho ES, Sharer LR, Barnett MH, Oleszak EL, Hoffman B, Morgan BP (2001) Immunopathology of secondary-progressive multiple sclerosis. Ann Neurol 50(5):646–657
110. Marin-Teva JL, Cuadros MA, Martin-Oliva D, Navascues J (2011) Microglia and neuronal cell death. Neuron Glia Biol 7(1):25–40. doi:10.1017/S1740925X12000014
111. Raivich G, Banati R (2004) Brain microglia and blood-derived macrophages: molecular profiles and functional roles in multiple sclerosis and animal models of autoimmune demyelinating disease. Brain Res Brain Res Rev 46(3):261–281. doi:10.1016/j.brainresrev.2004.06.006
112. Derkow K, Kruger C, Dembny P, Lehnardt S (2015) Microglia induce neurotoxic IL-17+ gammadelta T cells dependent on TLR2, TLR4, and TLR9 activation. PLoS One 10(8):e0135898. doi:10.1371/journal.pone.0135898
113. Almolda B, Gonzalez B, Castellano B (2015) Are microglial cells the regulators of lymphocyte responses in the CNS? Front Cell Neurosci 9:440. doi:10.3389/fncel.2015.00440
114. Heppner FL, Greter M, Marino D, Falsig J, Raivich G, Hovelmeyer N, Waisman A, Rulicke T et al (2005) Experimental autoimmune encephalomyelitis repressed by microglial paralysis. Nat Med 11(2):146–152. doi:10.1038/nm1177
115. Zhou J, Cai W, Jin M, Xu J, Wang Y, Xiao Y, Hao L, Wang B et al (2015) 18Beta-glycyrrhetinic acid suppresses experimental autoimmune encephalomyelitis through inhibition of microglia activation and promotion of remyelination. Sci Rep 5:13713. doi:10.1038/srep13713
116. Muja N, Cohen ME, Zhang J, Kim H, Gilad AA, Walczak P, Ben-Hur T, Bulte JW (2011) Neural precursors exhibit distinctly different patterns of cell migration upon transplantation during either the acute or chronic phase of EAE: a serial MR imaging study. Magn Reson Med 65(6):1738–1749. doi:10.1002/mrm.22757
117. Aarum J, Sandberg K, Haeberlein SL, Persson MA (2003) Migration and differentiation of neural precursor cells can be directed by microglia. Proc Natl Acad Sci U S A 100(26):15983–15988. doi:10.1073/pnas.2237050100
118. Cohen ME, Fainstein N, Lavon I, Ben-Hur T (2014) Signaling through three chemokine receptors triggers the migration of transplanted neural precursor cells in a model of multiple sclerosis. Stem Cell Res 13(2):227–239. doi:10.1016/j.scr.2014.06.001
119. Liu J, Hjorth E, Zhu M, Calzarossa C, Samuelsson EB, Schultzberg M, Akesson E (2013) Interplay between human microglia and neural stem/progenitor cells in an allogeneic co-culture model. J Cell Mol Med 17(11):1434–1443. doi:10.1111/jcmm.12123
120. Butovsky O, Ziv Y, Schwartz A, Landa G, Talpalar AE, Pluchino S, Martino G, Schwartz M (2006) Microglia activated by IL-4 or IFN-gamma differentially induce neurogenesis and oligodendrogenesis from adult stem/progenitor cells. Mol Cell Neurosci 31(1):149–160. doi:10.1016/j.mcn.2005.10.006
121. Guadagno J, Xu X, Karajgikar M, Brown A, Cregan SP (2013) Microglia-derived TNFalpha induces apoptosis in neural precursor cells via transcriptional activation of the Bcl-2 family member Puma. Cell Death Dis 4:e538. doi:10.1038/cddis.2013.59
122. Wu HM, Zhang LF, Ding PS, Liu YJ, Wu X, Zhou JN (2014) Microglial activation mediates host neuronal survival induced by neural stem cells. J Cell Mol Med 18(7):1300–1312. doi:10.1111/jcmm.12281
123. Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G et al (2006) Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci 9(7):917–924. doi:10.1038/nn1715
124. Takahashi K, Rochford CD, Neumann H (2005) Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. J Exp Med 201(4):647–657. doi:10.1084/jem.20041611
125. Mosher KI, Andres RH, Fukuhara T, Bieri G, Hasegawa-Moriyama M, He Y, Guzman R, Wyss-Coray T (2012) Neural progenitor cells regulate microglia functions and activity. Nat Neurosci 15(11):1485–1487. doi:10.1038/nn.3233

126. Sakata H, Niizuma K, Yoshioka H, Kim GS, Jung JE, Katsu M, Narasimhan P, Maier CM et al (2012) Minocycline-preconditioned neural stem cells enhance neuroprotection after ischemic stroke in rats. J Neurosci 32(10):3462–3473. doi:10.1523/jneurosci.5686-11.2012
127. Rasmussen S, Imitola J, Ayuso-Sacido A, Wang Y, Starossom SC, Kivisakk P, Zhu B, Meyer M et al (2011) Reversible neural stem cell niche dysfunction in a model of multiple sclerosis. Ann Neurol 69(5):878–891. doi:10.1002/ana.22299
128. Reubinoff BE, Itsykson P, Turetsky T, Pera MF, Reinhartz E, Itzik A, Ben-Hur T (2001) Neural progenitors from human embryonic stem cells. Nat Biotechnol 19(12):1134–1140. doi:10.1038/nbt1201-1134
129. Nait-Oumesmar B, Picard-Riera N, Kerninon C, Baron-Van Evercooren A (2008) The role of SVZ-derived neural precursors in demyelinating diseases: from animal models to multiple sclerosis. J Neurol Sci 265(1–2):26–31. doi:10.1016/j.jns.2007.09.032
130. Hermann A, Gastl R, Liebau S, Popa MO, Fiedler J, Boehm BO, Maisel M, Lerche H et al (2004) Efficient generation of neural stem cell-like cells from adult human bone marrow stromal cells. J Cell Sci 117(Pt 19):4411–4422. doi:10.1242/jcs.01307
131. Yang J, Yan Y, Ciric B, Yu S, Guan Y, Xu H, Rostami A, Zhang GX (2010) Evaluation of bone marrow- and brain-derived neural stem cells in therapy of central nervous system autoimmunity. Am J Pathol 177(4):1989–2001. doi:10.2353/ajpath.2010.091203
132. Hew M, O'Connor K, Edel MJ, Lucas M (2015) The possible future roles for iPSC-derived therapy for autoimmune diseases. J Clin Med 4(6):1193–1206. doi:10.3390/jcm4061193
133. Liu J (2013) Induced pluripotent stem cell-derived neural stem cells: new hope for stroke? Stem Cell Res Ther 4(5):115. doi:10.1186/scrt326
134. Yan Y, Shin S, Jha BS, Liu Q, Sheng J, Li F, Zhan M, Davis J et al (2013) Efficient and rapid derivation of primitive neural stem cells and generation of brain subtype neurons from human pluripotent stem cells. Stem Cells Transl Med 2(11):862–870. doi:10.5966/sctm.2013-0080
135. Wang T, Choi E, Monaco MC, Campanac E, Medynets M, Do T, Rao P, Johnson KR et al (2013) Derivation of neural stem cells from human adult peripheral CD34+ cells for an autologous model of neuroinflammation. PLoS One 8(11):e81720. doi:10.1371/journal.pone.0081720
136. Mozafari S, Laterza C, Roussel D, Bachelin C, Marteyn A, Deboux C, Martino G, Baron-Van Evercooren A (2015) Skin-derived neural precursors competitively generate functional myelin in adult demyelinated mice. J Clin Invest 125(9):3642–3656. doi:10.1172/JCI80437
137. Tao GZ, Lehwald N, Jang KY, Baek J, Xu B, Omary MB, Sylvester KG (2013) Wnt/beta-catenin signaling protects mouse liver against oxidative stress-induced apoptosis through the inhibition of forkhead transcription factor FoxO3. J Biol Chem 288(24):17214–17224. doi:10.1074/jbc.M112.445965
138. Nicaise AM, Banda E, Guzzo RM, Russomanno K, Castro-Borrero W, Willis CM, Johnson KM, Lo AC et al (2016) iPS-derived neural progenitor cells from PPMS patients reveal defect in myelin injury response. Exp Neurol 288:114–121. doi:10.1016/j.expneurol.2016.11.012
139. Lee ST, Chu K, Jung KH, Song YM, Jeon D, Kim SU, Kim M, Lee SK et al (2011) Direct generation of neurosphere-like cells from human dermal fibroblasts. PLoS One 6(7):e21801. doi:10.1371/journal.pone.0021801
140. Capetian P, Azmitia L, Pauly MG, Krajka V, Stengel F, Bernhardi EM, Klett M, Meier B et al (2016) Plasmid-based generation of induced neural stem cells from adult human fibroblasts. Front Cell Neurosci 10:245. doi:10.3389/fncel.2016.00245
141. Thier M, Worsdorfer P, Lakes YB, Gorris R, Herms S, Opitz T, Seiferling D, Quandel T et al (2012) Direct conversion of fibroblasts into stably expandable neural stem cells. Cell Stem Cell 10(4):473–479. doi:10.1016/j.stem.2012.03.003
142. Mirakhori F, Zeynali B, Rassouli H, Shahbazi E, Hashemizadeh S, Kiani S, Salekdeh GH, Baharvand H (2015) Induction of neural progenitor-like cells from human fibroblasts via a genetic material-free approach. PLoS One 10(8):e0135479. doi:10.1371/journal.pone.0135479
143. Miura T, Sugawara T, Fukuda A, Tamoto R, Kawasaki T, Umezawa A, Akutsu H (2015) Generation of primitive neural stem cells from human fibroblasts using a defined set of factors. Biol Open 4(11):1595–1607. doi:10.1242/bio.013151
144. Lujan E, Chanda S, Ahlenius H, Sudhof TC, Wernig M (2012) Direct conversion of mouse fibroblasts to self-renewing, tripotent neural precursor cells. Proc Natl Acad Sci U S A 109(7):2527–2532. doi:10.1073/pnas.1121003109
145. Plaisted WC, Zavala A, Hingco E, Tran H, Coleman R, Lane TE, Loring JF, Walsh CM (2016) Remyelination is correlated with regulatory T cell induction following human embryoid body-derived neural precursor cell transplantation in a viral model of multiple sclerosis. PLoS One 11(6):e0157620. doi:10.1371/journal.pone.0157620
146. Hawryluk GW, Mothe AJ, Chamankhah M, Wang J, Tator C, Fehlings MG (2012) In vitro characterization of trophic factor expression in neural precursor cells. Stem Cells Dev 21(3):432–447. doi:10.1089/scd.2011.0242
147. Fainstein N, Einstein O, Cohen ME, Brill L, Lavon I, Ben-Hur T (2013) Time limited immunomodulatory functions of transplanted neural precursor cells. Glia 61(2):140–149. doi:10.1002/glia.22420
148. Martino G, Franklin RJ, Baron Van Evercooren A, Kerr DA, Stem Cells in Multiple Sclerosis Consensus G (2010) Stem cell transplantation in multiple sclerosis: current status and future prospects. Nat Rev Neurol 6(5):247–255. doi:10.1038/nrneurol.2010.35
149. Einstein O, Fainstein N, Vaknin I, Mizrachi-Kol R, Reihartz E, Grigoriadis N, Lavon I, Baniyash M et al (2007) Neural precursors attenuate autoimmune encephalomyelitis by peripheral immunosuppression. Ann Neurol 61(3):209–218. doi:10.1002/ana.21033
150. Pluchino S, Zanotti L, Brambilla E, Rovere-Querini P, Capobianco A, Alfaro-Cervello C, Salani G, Cossetti C et al (2009) Immune regulatory neural stem/precursor cells protect from central nervous system autoimmunity by restraining dendritic cell function. PLoS One 4(6):e5959. doi:10.1371/journal.pone.0005959
151. Constantinescu CS, Farooqi N, O'Brien K, Gran B (2011) Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol 164(4):1079–1106. doi:10.1111/j.1476-5381.2011.01302.x
152. Donega M, Giusto E, Cossetti C, Schaeffer J, Pluchino S (2014) Systemic injection of neural stem/progenitor cells in mice with chronic EAE. J Vis Exp 86. doi:10.3791/51154
153. Pluchino S, Quattrini A, Brambilla E, Gritti A, Salani G, Dina G, Galli R, Del Carro U et al (2003) Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. Nature 422(6933):688–694. doi:10.1038/nature01552
154. Ben-Hur T, Einstein O, Mizrachi-Kol R, Ben-Menachem O, Reinhartz E, Karussis D, Abramsky O (2003) Transplanted multipotential neural precursor cells migrate into the inflamed white matter in response to experimental autoimmune encephalomyelitis. Glia 41(1):73–80. doi:10.1002/glia.10159
155. Wu S, Li K, Yan Y, Gran B, Han Y, Zhou F, Guan YT, Rostami A et al (2013) Intranasal delivery of neural stem cells: a CNS-specific, non-invasive cell-based therapy for experimental autoimmune encephalomyelitis. J Clin Cell Immunol 4(3). doi:10.4172/2155-9899.1000142

156. Guzman R, De Los AA, Cheshier S, Choi R, Hoang S, Liauw J, Schaar B, Steinberg G (2008) Intracarotid injection of fluorescence activated cell-sorted CD49d-positive neural stem cells improves targeted cell delivery and behavior after stroke in a mouse stroke model. Stroke 39(4):1300–1306. doi:10.1161/STROKEAHA.107.500470
157. Kokaia Z, Martino G, Schwartz M, Lindvall O (2012) Cross-talk between neural stem cells and immune cells: the key to better brain repair? Nat Neurosci 15(8):1078–1087. doi:10.1038/nn.3163
158. Martino G, Pluchino S (2006) The therapeutic potential of neural stem cells. Nat Rev Neurosci 7(5):395–406. doi:10.1038/nrn1908
159. Harris VK, Faroqui R, Vyshkina T, Sadiq SA (2012) Characterization of autologous mesenchymal stem cell-derived neural progenitors as a feasible source of stem cells for central nervous system applications in multiple sclerosis. Stem Cells Transl Med 1(7):536–547. doi:10.5966/sctm.2012-0015
160. Chen L, Coleman R, Leang R, Tran H, Kopf A, Walsh CM, Sears-Kraxberger I, Steward O et al (2014) Human neural precursor cells promote neurologic recovery in a viral model of multiple sclerosis. Stem Cell Reports 2(6):825–837. doi:10.1016/j.stemcr.2014.04.005
161. Di Ruscio A, Patti F, Welner RS, Tenen DG, Amabile G (2015) Multiple sclerosis: getting personal with induced pluripotent stem cells. Cell Death Dis 6:e1806. doi:10.1038/cddis.2015.179
162. Ravanidis S, Poulatsidou KN, Lagoudaki R, Touloumi O, Polyzoidou E, Lourbopoulos A, Nousiopoulou E, Theotokis P et al (2015) Subcutaneous transplantation of neural precursor cells in experimental autoimmune encephalomyelitis reduces chemotactic signals in the central nervous system. Stem Cells Transl Med 4(12):1450–1462. doi:10.5966/sctm.2015-0068
163. Pluchino S, Zanotti L, Rossi B, Brambilla E, Ottoboni L, Salani G, Martinello M, Cattalini A et al (2005) Neurosphere-derived multipotent precursors promote neuroprotection by an immunomodulatory mechanism. Nature 436(7048):266–271. doi:10.1038/nature03889
164. Einstein O, Karussis D, Grigoriadis N, Mizrachi-Kol R, Reinhartz E, Abramsky O, Ben-Hur T (2003) Intraventricular transplantation of neural precursor cell spheres attenuates acute experimental allergic encephalomyelitis. Mol Cell Neurosci 24(4):1074–1082
165. Whitman LM, Blanc CA, Schaumburg CS, Rowitch DH, Lane TE (2012) Olig1 function is required for remyelination potential of transplanted neural progenitor cells in a model of viral-induced demyelination. Exp Neurol 235(1):380–387. doi:10.1016/j.expneurol.2012.03.003
166. Einstein O, Friedman-Levi Y, Grigoriadis N, Ben-Hur T (2009) Transplanted neural precursors enhance host brain-derived myelin regeneration. J Neurosci 29(50):15694–15702. doi:10.1523/JNEUROSCI.3364-09.2009
167. Harris VK, Yan OJ, Vyshkina T, Sahabi S, Liu X, Sadiq SA (2012) Clinical and pathological effects of intrathecal injection of mesenchymal stem cell-derived neural progenitors in an experimental model of multiple sclerosis. J Neurol Sci 313(1–2):167–177. doi:10.1016/j.jns.2011.08.036
168. Laterza C, Merlini A, De Feo D, Ruffini F, Menon R, Onorati M, Fredrickx E, Muzio L et al (2013) iPSC-derived neural precursors exert a neuroprotective role in immune-mediated demyelination via the secretion of LIF. Nat Commun 4:2597. doi:10.1038/ncomms3597
169. Reekmans K, Praet J, De Vocht N, Daans J, Van der Linden A, Berneman Z, Ponsaerts P (2012) Stem cell therapy for multiple sclerosis: preclinical evidence beyond all doubt? Regen Med 7(2):245–259. doi:10.2217/rme.12.5
170. Ricci-Vitiani L, Lombardi DG, Signore M, Biffoni M, Pallini R, Parati E, Peschle C, De Maria R (2007) Human neural progenitor cells display limited cytotoxicity and increased oligodendrogenesis during inflammation. Cell Death Differ 14(4):876–878. doi:10.1038/sj.cdd.4402078
171. Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nat Rev Neurosci 9(11):839–855. doi:10.1038/nrn2480
172. Walker MR, Patel KK, Stappenbeck TS (2009) The stem cell niche. J Pathol 217(2):169–180. doi:10.1002/path.2474
173. Ben-Hur T, Ben-Menachem O, Furer V, Einstein O, Mizrachi-Kol R, Grigoriadis N (2003) Effects of proinflammatory cytokines on the growth, fate, and motility of multipotential neural precursor cells. Mol Cell Neurosci 24(3):623–631
174. Foote AK, Blakemore WF (2005) Inflammation stimulates remyelination in areas of chronic demyelination. Brain 128(Pt 3):528–539. doi:10.1093/brain/awh417
175. Molina-Holgado E, Vela JM, Arevalo-Martin A, Guaza C (2001) LPS/IFN-gamma cytotoxicity in oligodendroglial cells: role of nitric oxide and protection by the anti-inflammatory cytokine IL-10. Eur J Neurosci 13(3):493–502
176. Perez-Asensio FJ, Perpina U, Planas AM, Pozas E (2013) Interleukin-10 regulates progenitor differentiation and modulates neurogenesis in adult brain. J Cell Sci 126(Pt 18):4208–4219. doi:10.1242/jcs.127803
177. Kulkarni A, Scully TJ, O'Donnell LA (2016) The antiviral cytokine interferon-gamma restricts neural stem/progenitor cell proliferation through activation of STAT1 and modulation of retinoblastoma protein phosphorylation. J Neurosci Res. doi:10.1002/jnr.23987
178. Kizil C, Kyritsis N, Brand M (2015) Effects of inflammation on stem cells: together they strive? EMBO Rep 16(4):416–426. doi:10.15252/embr.201439702
179. Liu Q, Sanai N, Jin WN, La Cava A, Van Kaer L, Shi FD (2016) Neural stem cells sustain natural killer cells that dictate recovery from brain inflammation. Nat Neurosci 19(2):243–252. doi:10.1038/nn.4211
180. Giannakopoulou A, Grigoriadis N, Polyzoidou E, Lourbopoulos A, Michaloudi E, Papadopoulos GC (2011) Time-dependent fate of transplanted neural precursor cells in experimental autoimmune encephalomyelitis mice. Exp Neurol 230(1):16–26. doi:10.1016/j.expneurol.2010.04.011
181. Mueller FJ, Serobyan N, Schraufstatter IU, DiScipio R, Wakeman D, Loring JF, Snyder EY, Khaldoyanidi SK (2006) Adhesive interactions between human neural stem cells and inflamed human vascular endothelium are mediated by integrins. Stem Cells 24(11):2367–2372. doi:10.1634/stemcells.2005-0568
182. Imitola J, Raddassi K, Park KI, Mueller FJ, Nieto M, Teng YD, Frenkel D, Li J et al (2004) Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1alpha/CXC chemokine receptor 4 pathway. Proc Natl Acad Sci U S A 101(52):18117–18122. doi:10.1073/pnas.0408258102
183. Carbajal KS, Schaumburg C, Strieter R, Kane J, Lane TE (2010) Migration of engrafted neural stem cells is mediated by CXCL12 signaling through CXCR4 in a viral model of multiple sclerosis. Proc Natl Acad Sci U S A 107(24):11068–11073. doi:10.1073/pnas.1006375107
184. Nait-Oumesmar B, Picard-Riera N, Kerninon C, Decker L, Seilhean D, Hoglinger GU, Hirsch EC, Reynolds R et al (2007) Activation of the subventricular zone in multiple sclerosis: evidence for early glial progenitors. Proc Natl Acad Sci U S A 104(11):4694–4699. doi:10.1073/pnas.0606835104
185. Abdanipour A, Sagha M, Noori-Zadeh A, Pakzad I, Tiraihi T (2015) In vitro study of the long-term cortisol treatment effects on the growth rate and proliferation of the neural stem/precursor cells. Neurol Res 37(2):117–124. doi:10.1179/1743132814y.0000000431
186. Pluchino S, Muzio L, Imitola J, Deleidi M, Alfaro-Cervello C, Salani G, Porcheri C, Brambilla E et al (2008) Persistent

inflammation alters the function of the endogenous brain stem cell compartment. Brain 131(Pt 10):2564–2578. doi:10.1093/brain/awn198
187. Dooley D, Vidal P, Hendrix S (2014) Immunopharmacological intervention for successful neural stem cell therapy: new perspectives in CNS neurogenesis and repair. Pharmacol Ther 141(1):21–31. doi:10.1016/j.pharmthera.2013.08.001
188. Girard C, Bemelmans AP, Dufour N, Mallet J, Bachelin C, Nait-Oumesmar B, Baron-Van Evercooren A, Lachapelle F (2005) Grafts of brain-derived neurotrophic factor and neurotrophin 3-transduced primate Schwann cells lead to functional recovery of the demyelinated mouse spinal cord. J Neurosci 25(35):7924–7933. doi:10.1523/JNEUROSCI.4890-04.2005
189. Neri M, Maderna C, Ferrari D, Cavazzin C, Vescovi AL, Gritti A (2010) Robust generation of oligodendrocyte progenitors from human neural stem cells and engraftment in experimental demyelination models in mice. PLoS One 5(4):e10145. doi:10.1371/journal.pone.0010145
190. Yang J, Yan Y, Xia Y, Kang T, Li X, Ciric B, Xu H, Rostami A et al (2014) Neurotrophin 3 transduction augments remyelinating and immunomodulatory capacity of neural stem cells. Mol Ther 22(2):440–450. doi:10.1038/mt.2013.241
191. Gao X, Deng L, Wang Y, Yin L, Yang C, Du J, Yuan Q (2016) GDNF enhances therapeutic efficiency of neural stem cells-based therapy in chronic experimental allergic encephalomyelitis in rat. Stem Cells Int 2016:1431349. doi:10.1155/2016/1431349
192. Linker RA, Lee DH, Demir S, Wiese S, Kruse N, Siglienti I, Gerhardt E, Neumann H et al (2010) Functional role of brain-derived neurotrophic factor in neuroprotective autoimmunity: therapeutic implications in a model of multiple sclerosis. Brain 133(Pt 8):2248–2263. doi:10.1093/brain/awq179
193. Cao W, Yang Y, Wang Z, Liu A, Fang L, Wu F, Hong J, Shi Y et al (2011) Leukemia inhibitory factor inhibits T helper 17 cell differentiation and confers treatment effects of neural progenitor cell therapy in autoimmune disease. Immunity 35(2):273–284. doi:10.1016/j.immuni.2011.06.011
194. Ma H, Yu B, Kong L, Zhang Y, Shi Y (2012) Neural stem cells over-expressing brain-derived neurotrophic factor (BDNF) stimulate synaptic protein expression and promote functional recovery following transplantation in rat model of traumatic brain injury. Neurochem Res 37(1):69–83. doi:10.1007/s11064-011-0584-1
195. Chang DJ, Lee N, Choi C, Jeon I, Oh SH, Shin DA, Hwang TS, Lee HJ et al (2013) Therapeutic effect of BDNF-overexpressing human neural stem cells (HB1.F3.BDNF) in a rodent model of middle cerebral artery occlusion. Cell Transplant 22(8):1441–1452. doi:10.3727/096368912X657323
196. Kandalam S, Sindji L, Delcroix GJ, Violet F, Garric X, Andre EM, Schiller PC, Venier-Julienne MC (2016) Pharmacologically active microcarriers delivering BDNF within a hydrogel: novel strategy for human bone marrow-derived stem cells neural/neuronal differentiation guidance and therapeutic secretome enhancement. Acta Biomater. doi:10.1016/j.actbio.2016.11.030
197. Klose J, Schmidt NO, Melms A, Dohi M, Miyazaki J, Bischof F, Greve B (2013) Suppression of experimental autoimmune encephalomyelitis by interleukin-10 transduced neural stem/progenitor cells. J Neuroinflammation 10:117. doi:10.1186/1742-2094-10-117
198. Yang J, Jiang Z, Fitzgerald DC, Ma C, Yu S, Li H, Zhao Z, Li Y et al (2009) Adult neural stem cells expressing IL-10 confer potent immunomodulation and remyelination in experimental autoimmune encephalitis. J Clin Invest 119(12):3678–3691. doi:10.1172/JCI37914
199. Gudi V, Skuljec J, Yildiz O, Frichert K, Skripuletz T, Moharregh-Khiabani D, Voss E, Wissel K et al (2011) Spatial and temporal profiles of growth factor expression during CNS demyelination reveal the dynamics of repair priming. PLoS One 6(7):e22623. doi:10.1371/journal.pone.0022623
200. Mason JL, Ye P, Suzuki K, D'Ercole AJ, Matsushima GK (2000) Insulin-like growth factor-1 inhibits mature oligodendrocyte apoptosis during primary demyelination. J Neurosci 20(15):5703–5708
201. Sabo JK, Aumann TD, Kilpatrick TJ, Cate HS (2013) Investigation of sequential growth factor delivery during cuprizone challenge in mice aimed to enhance oligodendrogliogenesis and myelin repair. PLoS One 8(5):e63415. doi:10.1371/journal.pone.0063415
202. Shi B, Ding J, Liu Y, Zhuang X, Zhuang X, Chen X, Fu C (2014) ERK1/2 pathway-mediated differentiation of IGF-1-transfected spinal cord-derived neural stem cells into oligodendrocytes. PLoS One 9(8):e106038. doi:10.1371/journal.pone.0106038
203. Lee YE, An J, Lee KH, Kim SS, Song HJ, Pyeon H, Nam H, Kang K et al (2016) Correction: The synergistic local immunosuppressive effects of neural stem cells expressing Indoleamine 2,3-dioxygenase (IDO) in an experimental autoimmune encephalomyelitis (EAE) animal model. PLoS One 11(2):e0148720. doi:10.1371/journal.pone.0148720
204. Sher F, Amor S, Gerritsen W, Baker D, Jackson SL, Boddeke E, Copray S (2012) Intraventricularly injected Olig2-NSCs attenuate established relapsing-remitting EAE in mice. Cell Transplant 21(9):1883–1897. doi:10.3727/096368911X637443
205. Zhou Q, Anderson DJ (2002) The bHLH transcription factors OLIG2 and OLIG1 couple neuronal and glial subtype specification. Cell 109(1):61–73
206. Geurts JJ, Bo L, Roosendaal SD, Hazes T, Daniels R, Barkhof F, Witter MP, Huitinga I et al (2007) Extensive hippocampal demyelination in multiple sclerosis. J Neuropathol Exp Neurol 66(9):819–827. doi:10.1097/nen.0b013e3181461f54
207. Braun SM, Pilz GA, Machado RA, Moss J, Becher B, Toni N, Jessberger S (2015) Programming hippocampal neural stem/progenitor cells into oligodendrocytes enhances remyelination in the adult brain after injury. Cell Rep 11(11):1679–1685. doi:10.1016/j.celrep.2015.05.024
208. Mi S, Lee X, Shao Z, Thill G, Ji B, Relton J, Levesque M, Allaire N et al (2004) LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. Nat Neurosci 7(3):221–228. doi:10.1038/nn1188
209. Li X, Zhang Y, Yan Y, Ciric B, Ma CG, Gran B, Curtis M, Rostami A et al (2016) Neural stem cells engineered to express three therapeutic factors mediate recovery from chronic stage CNS autoimmunity. Mol Ther 24(8):1456–1469. doi:10.1038/mt.2016.104
210. Snyder EY, Deitcher DL, Walsh C, Arnold-Aldea S, Hartwieg EA, Cepko CL (1992) Multipotent neural cell lines can engraft and participate in development of mouse cerebellum. Cell 68(1):33–51
211. Kim SU (2007) Genetically engineered human neural stem cells for brain repair in neurological diseases. Brain Dev 29(4):193–201. doi:10.1016/j.braindev.2006.07.012
212. Hackett C, Knight J, Mao-Draayer Y (2014) Transplantation of Fas-deficient or wild-type neural stem/progenitor cells (NPCs) is equally efficient in treating experimental autoimmune encephalomyelitis (EAE). Am J Transl Res 6(2):119–128
213. Glass JD, Hertzberg VS, Boulis NM, Riley J, Federici T, Polak M, Bordeau J, Fournier C et al (2016) Transplantation of spinal cord-derived neural stem cells for ALS: analysis of phase 1 and 2 trials. Neurology 87(4):392–400. doi:10.1212/WNL.0000000000002889
214. Harris VK, Vyshkina T, Sadiq SA (2016) Clinical safety of intrathecal administration of mesenchymal stromal cell-derived neural progenitors in multiple sclerosis. Cytotherapy 18(12):1476–1482. doi:10.1016/j.jcyt.2016.08.007
215. Gupta N, Henry RG, Strober J, Kang SM, Lim DA, Bucci M, Caverzasi E, Gaetano L et al (2012) Neural stem cell engraftment and myelination in the human brain. Sci Transl Med 4(155):155ra137. doi:10.1126/scitranslmed.3004373