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Journal
Expert Review of Neurotherapeutics, 14(10)

ISSN
1473-7175

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Publication Date
2014-10-01

DOI
10.1586/14737175.2014.955854

Peer reviewed

Published in final edited form as:
Expert Rev Neurother. 2014 October ; 14(10): 1169–1179. doi:10.1586/14737175.2014.955854.

Promoting remyelination: utilizing a viral model of demyelination to assess cell-based therapies

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS. While a broad range of therapeutics effectively reduce the incidence of focal white matter inflammation and plaque formation for patients with relapse-remitting forms of MS, a challenge within the field is to develop therapies that allow for axonal protection and remyelination. In the last decade, growing interest has focused on utilizing neural precursor cells (NPCs) to promote remyelination. To understand how NPCs function in chronic demyelinating environments, several excellent pre-clinical mouse models have been developed. One well accepted model is infection of susceptible mice with neurotropic variants of mouse hepatitis virus (MHV) that undergo chronic demyelination exhibiting clinical and histopathologic similarities to MS patients. Combined with the possibility that an environmental agent such as a virus could trigger MS, the MHV model of demyelination presents a relevant mouse model to assess the therapeutic potential of NPCs transplanted into an environment in which inflammatory-mediated demyelination is established.

Keywords

demyelination; multiple sclerosis; neural precursor cells; neural progenitor cells; remyelination; virus

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Financial & competing interests disclosure: The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Neural precursor cells (NPCs) represent heterogeneous, stem-like cells capable of self-renewal and multipotent differentiation potential within the developing and fully mature CNS [1]. Over 20 years ago, pioneering research demonstrated that NPCs could be isolated and cultured *ex vivo* from mitotically active regions within the embryonic rat and mouse and differentiate into neurons and glia [2,3]. Since then, stringent culturing protocols have been developed, enabling researchers to generate high-purity NPCs safely from various mammalian sources, including both embryonic and adult tissues [4–7]. The uncommitted nature of NPCs makes them a promising therapeutic candidate for the human demyelinating disease multiple sclerosis (MS). Not only do NPCs have the potential to replace damaged or nonfunctional cells within the CNS to promote repair and recovery, but they are also known to secrete immunomodulatory and neurotrophic factors, further expanding the therapeutic potential of the cells [8]. In order to assess the functional roles of engrafted NPCs, it is important to fully understand a broad range of inflammatory niches that may be supportive for NPC survival and function.

The mouse hepatitis virus (MHV) model of demyelination is a relevant MS model that differs from autoimmune-mediated demyelination including experimental autoimmune encephalomyelitis (EAE) as well as glial toxin models, for example, cuprizone, lysolecithin and ethidium bromide [9–12]. Mice infected with neurotrophic variants of MHV mice undergo chronic demyelination that is promoted through effector activity of virus-specific and nonspecific T cells [13,14]. Given the possibility of viral infection in initiating demyelination in humans as well as the fact that numerous neurotrophic viruses exist that are capable of persisting within the CNS, it is important to evaluate the therapeutic potential of engrafted NPCs in the presence of a persistent viral infection that is correlative with chronic neuroinflammation and demyelination [15].

Multiple sclerosis

MS is a complex disease of the CNS that is characterized by heterogeneous pathologies composed of both inflammatory and neurodegenerative components [16]. Although the identification of an etiological trigger of MS remains elusive, disease induction is thought to result from several features including both genetic predisposition and environmental factors, for example, microbial infection [17–22]. The most common histopathological feature at early stages of the disease includes intermittent episodes of acute inflammation within patches of white matter, resulting in demyelination [23]. Myelin is critical for maintaining efficient axonal conduction and oligodendrocytes, the myelin producer and maintainer of axonal health within the CNS, are damaged or destroyed in MS patients. Focal attacks during early disease are generally episodic, varying from 24 h to several weeks in length and are usually followed by near complete recovery of clinical symptoms, a disease course collectively referred to as relapse-remitting MS (RRMS) [24]. Spontaneous remission can be associated with waning inflammation and partial restoration of axonal conductivity due to remyelination [25,26]. Endogenous oligodendrocyte precursor cells (OPCs) are found to be universally dispersed within the human CNS and can be found in high density within some subacute lesions during early stages of MS [27]. Within a subacute demyelinating lesion, perivascular infiltrates composed of 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 [23,28]. Additionally, clonally expanded class I restricted CD8+ T cells are found in close proximity to demyelinated axons and potentially target myelin epitopes [29]. Remyelination following OPC maturation leads to the formation of shadow plaques, in which patches of remyelinated white matter are composed of disproportionally thin myelin sheaths surrounding axons [27,30–35].

Although RRMS can last throughout the individual's lifespan, approximately 80% of patients with RRMS will develop a progressive disease within two decades following diagnosis, whereas 15% of individuals diagnosed with MS are classified as progressive patients [36]. In general, progressive MS is the latest stage of the disease, characterized by a gradual worsening of symptoms without remission. Severe neurological impairments dramatically reduce the quality of life for the individual, and this is mainly attributed to expanding cortical lesions impacting motor function. Pathologically, there is widespread axonal degeneration and grey matter neuropathy without a significant presence of the adaptive arm of immunity contributing to the immunopathology [37–40]. Rather, diffuse white and gray matter inflammation has been reported, correlating, in part, to global microglial activation as well as the presence of T cells, B cells and myelin-laden macrophages, which are restricted to the borders of preexisting lesions [28,41]. Furthermore, there is an overall failure of OPCs to efficiently remyelinate damaged white and gray matter areas, dramatically reducing the possibility for recovery [26,42].

With the use of preclinical mouse models of demyelination to study MS, researchers have identified both antigenic and cellular components of the disease that are believed to contribute to demyelination. This has led to the development of therapeutic advances that have generated favorable long-term prognoses for patients. Many of these US FDA-approved therapies show potent immunosuppressive properties that act to limit leukocyte infiltration into the brain, thereby reducing relapse events [43]. However, for patients with progressive disease, the therapeutic repertoire and treatment decision making is confounding. This is partially reflected by the reduced efficacy of many drugs whose mode of action is to inhibit leukocyte infiltration to the CNS, rather than target cells implicated in accelerating progressive disease, such as reactive astrocytes, microglia and degenerating neurons. In addition, an intact blood–brain barrier (BBB) during progressive MS may compartmentalize inflammatory leukocytes within the CNS, making many drugs inactive due to their inability to penetrate a reconstituted BBB [44]. Most importantly for patients with progressive MS is the fact that there are no treatment options that act to halt or even slow demyelination and axonal loss.

One therapeutic option to treat progressive MS would be to replenish or rejuvenate the pool of endogenous OPCs that show limited remyelination potential in the later stages of disease. Among the plethora of experimental approaches attempting to promote remyelination and repair, cellular replacement therapies using NPCs have emerged as a clinically relevant area of research. Recent successes demonstrating that bone marrow-derived mesenchymal neural precursor cells derived from mouse and human showed therapeutic potential for promoting recovery in preclinical mouse MS models resulted in FDA-approved Phase I safety-feasibility clinical trials for progressive MS patients using autologous bone marrow-derived

mesenchymal neural precursor cells [45–48]. Moreover, transplantation of human fetal-derived NPCs into the frontal lobes of children with Pelizaeus–Merbacher disease, a rare dysmyelinating disorder in children, has revealed measurable gains in motor and/or cognition associated with remyelination [49]. Therefore, CNS delivery of hNPCs is a potentially viable and important approach to treat human demyelinating diseases such as MS.

A viral model of demyelination

Intracranial inoculation of C56BL/6 mice with the neurotrophic JHM strain of mouse hepatitis virus (JHMV) results in the dissemination of viral particles throughout the ventricular system before targeting ependymal cells lining the ventricles [50]. As virus penetrates further into the parenchyma of the brain and spinal cord, oligodendrocytes, astrocytes and microglia are susceptible to infection while neurons are sparred [51]. A robust innate host-defense response to JHM infection soon follows, characterized by the secretion of proinflammatory factors such as IFN-α, IFN-β, IL-1, IL-6, IL-12 and TNF-α [52,53]. Type I interferons have essential roles for protecting the host against JHM infection, as mice deficient in the IFN-α/β receptor show elevated viral load within the CNS and higher mortality, while exogenous treatment of mice with either Type I interferon limits dissemination of virus [54–56]. Furthermore, innate cellular components consisting of neutrophils, macrophages and natural killer (NK) cells respond to JHMV infection by rapidly migrating to the CNS to assist in permeabilizing the BBB [57–60]. BBB permeabilization promotes infiltration of sensitized JHMV-specific CD4+ and CD8+ T cells that can exert potent antiviral effector mechanisms [58,60,61].

Virus-specific CD4+ T cells function as a supporting cell for CD8+ T cells, promoting CD8+ T-cell expansion in the periphery and promoting survival and cytotoxicity within the CNS [62,63]. In addition, CD4+ T cells can control viral spread through their release of IFN-γ, which serves dual roles by inhibiting viral replication within oligodendrocytes and also inducing upregulation of MHC class II expression on microglia [62,64–67]. Depletion of CD4+ T cells alters CD8+ T cell-mediated control of viral replication within the CNS, mainly a result of reduced of IFN-γ expression and elevated CD8+ T-cell apoptosis [62]. Virus-specific CD8+ T cells are the primary cytolytic effector cell within the CNS during JHM infection and their peak accumulation coincides with viral clearance from glia [65,66,68]. *ex vivo* analysis of immunodominant JHMV-specific CD8+ T cells from JHMV-infected mice reveals elevated expression of IFN-γ and the cytolytic components granzyme B and perforin. *In vivo*, IFN-γ-mediated upregulation of MHC-I promotes perforin-mediated cytolysis of JHMV-infected astrocytes and microglia [64,68,69], while Fas/FasL and TNFR signaling on infected cells does not appear to contribute to viral clearance [70]. Oligodendrocytes are an initial reservoir for viral replication; however, they appear to be resistant to perforinmediated cytolysis by CD8+ T cells but rather control viral replication through IFN-γ receptor signaling. These findings are supported by Stohlman and colleagues [67], who demonstrated that expression of a dominant negative version of IFNGR on oligodendrocytes results in increased tropism and viral load within oligodendroglia throughout the brain and spinal cord.

Mice that survive acute JHMV infection progress into the immune-mediated chronic demyelinating phase of the disease, with clinical symptoms manifesting as ataxia and partial to complete hind limb paralysis, beginning 1 week following infection and peaking 2–3 weeks p.i. Histological cross sections of spinal cords from mice undergoing JHMV-induced demyelination demonstrate that oligodendrocyte dysfunction and loss of myelin integrity within white matter tracts is closely associated with the presence of both inflammatory leukocytes and presentation of viral antigen via MHC-I and MHC-II, rather than widespread apoptosis and/or necrosis of mature oligodendrocytes [71–73]. Moreover, a paucity of infectious viral particles within the CNS during chronic disease suggests that productive infection of new glial cells does not amplify demyelination. More likely, viral RNA quasispecies present within the CNS of persistently infected mice promote chronic inflammation and demyelination [74–76].

An effective way to visualize and quantify demyelination on a macroscopic scale is via luxol fast blue (LFB) staining. LFB contains a copper phthalocyanine dye that readily binds to lipoproteins found on myelin sheaths, resulting in a blue staining of myelin-rich regions and a lack of staining within demyelinating lesions. LFB analysis of spinal cord sections during persistent JHMV-infection reveals new lesion formation within the anterior funiculus of the spinal cord as early as day 7 p.i. [50]. As the severity neuroinflammation increases, new lesions are often observed within the lateral funiculus and posterior funiculus (Figure 1A) [50]. Additionally, axonopathy within the white matter tracts of the spinal cord is present as observed in the use of the SMI-32 or Bielschowsky's silver impregnation stain and initial observations suggested that this occurred concomitantly with demyelination, whereas axonal degeneration has been argued to precede oligodendrocyte dysregulation in MS [77,78].

Several studies have identified both T cells and macrophages as the main cellular component in inducing demyelination during chronic JHMV infection mice rather than viral-induced lysis of oligodendrocytes. This stems from studies showing that JHMV infection of *RAG1* -/- immunodeficient mice (lacking functional T- and B-lymphocytes) results in extensive viral replication within oligodendrocytes, but limited demyelination [72,79]. Moreover, adoptive transfer of JHMV-sensitized splenocytes from wild-type mice into JHMV-infected *RAG1*-/- mice results in demyelination, while adoptive transfer of enriched CD8+ T cells obtained from JHMV-sensitized IFN-γ-deficient mice into JHMV-infected *RAG1* -/- mice resulted in reduced severity of demyelination compared with recipients of IFN-γ-expressing CD8+ T cells, implicating IFN-γ expression from infiltrating CD8+ T cells as an important contributor to oligodendrocyte dysregulation [79]. Further studies utilizing *CD4* -/- or *CD8* -/- mice demonstrated the importance of both T-cell subsets in contributing to demyelination following JHMV infection [80]. With regard to IFN-γ impacting oligodendrocyte health, numerous reports have demonstrated enhanced sensitivity of OPCs to IFN-γ-induced apoptosis when compared with mature oligodendrocytes, suggesting that IFN-γ can induce cell death on oligodendrocyte-lineage cells depending on their maturation state [81–87]. This is supported by the finding that demyelination is observed within JHMV-infected mice whose selective ablation of the IFN-γ receptor on mature oligodendrocytes [67]. This could partially be explained by IFN-γ secretion by CD8+ T cells promoting the migration and accumulation of activated macrophages/microglia within white matter tracts of JHMV-

infected mice [72,79]. Indeed, ultrastructural and immunofluorescence analysis of JHMV-induced demyelinating lesions features macrophages/microglia engulfing myelin near demyelinated axons [88–90]. However, discriminating between these cells types by immunophenotyping and light microscopy remains a challenge to determine the exact role each play in contributing to pathology during chronic neurological disease. More recently, Ransohoff and colleagues [91] have used electron microscopy to show a more pathogenic role for macrophages compared with microglia in EAE model of MS. Epitope spreading and the appearance of autoreactive T cells against host neuroantigens are not thought to contribute to demyelination during chronic JHMV infection. Altogether, these findings suggest that demyelination is multifaceted and numerous factors could contribute to pathology.

Numerous experimental models of CNS injury have demonstrated that endogenous OPCs can respond to ongoing demyelination through proliferation and maturation, restoring depleted pools of mature oligodendrocytes and actively participating in CNS remyelination [92–97]. Following JHMV infection, PDGFR-positive OPCs found within the spinal cord increase sixfold between days 6 and 14 p.i., suggesting that OPCs become mitotically active following the onset of demyelination [98]. Additionally, a rebound in the total frequency of mature oligodendrocytes to preinfection levels by 7 weeks p.i is also observed, presumably due to the maturation of the expanding OPC population [98]. Nevertheless, OPC differentiation does not lead to substantial clinical recovery and full remyelination within persistently infected mice as exposed axons are still detected months later following infection (Figure 1B) [98].

Several studies have identified several cytokines, chemokines and growth factors that can impact OPC proliferation and maturation *in vivo* [96,98–100]. For example, signaling through the CXCR2 chemokine receptor has been shown to enhance OPC proliferation and aid in the directional migration of OPCs within the developing mouse spinal cord, while its inhibition resulted in reduced proliferation and increased maturation in an autoimmune model of demyelination [99–101]. Using the A59 variant of MHV, Armstrong and colleagues [100] demonstrated that PDGF and FGF2 can regulate OPC biology by stimulating proliferation and limiting maturation through activation of the notch signaling pathway. Within the context of JHMV-induced neurological disease, the CXCR4/CXCL12 signaling axis may also play a crucial role in aiding OPC maturation. Indeed, blocking CXCL12's ability to bind to CXCR4 using the small molecule AMD3100 leads to an increase in PDGFRa-positive OPCs and a decrease in mature oligodendrocytes within the spinal cord, suggesting that CXCR4 signaling promotes OPC differentiation. These results are supported and extended by Klein and colleagues [102], who showed that activation of the CXCL12 scavenger receptor CXCR7 as well as CXCR4 results in OPC maturating during cuprizone-induced demyelination.

Neural precursor cell engraftment in MHV-infected mice

To better understand the potential therapeutic roles for NPCs intraspinally engrafted into a demyelinating environment, we chose day 14 post-JHMV infection as animals that survived the acute stage of disease are persistently infected with virus and clinical disease that is

congruent with neuroinflammation and demyelination. To study the migratory and functional roles of NPCs without the possibility of rejection, early studies utilized a syngeneic transplant protocol whereby H-2b haplotype-matched mouse striatal NPCs from postnatal day 1 (P1) C56BL/6 mice were transplanted intraspinally into the T8 region of C57BL/6 recipient mice undergoing JHMV-induced demyelination [103]. Initial results demonstrated that transplanted NPCs readily migrated up to 12 mm both rostral and caudal from the transplant site as identified by colocalization of the oligodendrocyte marker CC1 and the proliferation marker Brdu [103]. Quantification of remyelinated axons resulted in up to 67% of axons remyelinated compared with 10% for nontransplanted controls, suggesting that NPCs can survive within the inflammatory niche and functionally incorporate throughout demyelinated white matter tracts following differentiation into mature oligodendrocytes [103]. Additional studies by Carbajal *et al*. [104] demonstrated that transplanted GFP-NPCs were shown to selectively colonize demyelinated white matter regions within the ventral and lateral funiculus regions of the spinal cord. Positional migration of NPCs was mediated, in part, by responding the CXC chemokine ligand CXCL12 via expression of the receptor CXCR4 expressed upon engrafted NPCs [104].

To determine whether reduction in clinical disease was due to the cells modifying the inflammatory microenvironment to reduce overall inflammation and increase recovery, mice that received mouse P1 NPCs were sacrificed at 10 and 14 days post-transplant and leukocyte infiltration was assessed by flow cytometry [105]. NPC transplantation did not alter the accumulation of T cells or macrophages within the CNS nor proinflammatory chemokine and cytokine gene expression, suggesting that the enhanced remyelination and recovery following transplantation was not a result of NPC bystander effects attenuating the inflammatory response [105].

An important question related to NPC transplantation is whether engrafted cells are capable of directly remyelinating axons or create a ‘nursing’ effect by secreting trophic factors capable of promoting maturation of endogenous OPCs into myelinating oligodendrocytes. We evaluated the contributions of the transcription factor OLIG1 on NPC differentiation and remyelination [106]. Under defined conditions, NPCs preferentially differentiate into oligodendroglia, whereas NPCs isolated from OLIG1-deficient (*Olig1*-/-) mice exhibit enhanced differentiation into astrocytes. Transplantation of *Olig1*-/- and *Olig1* +/+ NPCs into JHMV-infected mice resulted in similar cell survival, proliferation and selective migration to areas of demyelination. However, only recipients of wild-type NPCs exhibited extensive remyelination compared with mice receiving *Olig1*-/- NPCs. *In vivo* characterization of NPCs revealed that *Olig1*+/+ NPCs preferentially differentiated into NG2-positive OPCs and formed processes expressing myelin basic protein that encircled axons. In contrast, the majority of transplanted *Olig1*-/- NPCs differentiated into GFAP-positive cells consistent with the astrocyte lineage. These findings reveal that OLIG1 function is required for the remyelination potential of NPCs after transplant, through specification and/or maintenance of oligodendroglial identity. In addition, we have recently employed two-photon microscopy to assess intercellular interactions of transplanted NPCs *ex vivo* [107]. Within this model, JHMV-infected Thy1-YFP mice, which express yellow fluorescent protein (YFP) from medium-to-large caliber axons within the spinal cord,

received SVZ-derived NPCs that express GFP following their differentiation into oligodendrocytes (PLP-GFP). Several important observations were derived from this study including the finding that JHMV-infected Thy1-YFP mice displayed extensive axonal damage earlier than expected during JHMV-induced disease, suggesting that appearance of axonopathy precedes robust immune-mediated demyelination. This argues that axonal damage may be important in contributing to white matter damage and myelin loss. Whether viral infection of neurons and/or transport of viral proteins along axons is important in this process is currently not well defined [78]. In addition, two-photon imaging clearly showed that engrafted NPCs interacted with damaged axons and this resulted in remyelination and improved axonal integrity as determined by YFP expression (Figures 2A–2D) [107].

As an additional step to better understand the therapeutic potential of engraftment of NPCs in promoting clinical and histological recovery, we have transplanted MHC-mismatched mouse NPCs into JHMV-infected mice with established demyelination to determine whether allogeneic NPCs are recognized as foreign and rejected via immunological mechanisms. This is clinically relevant as transplantation of human NPCs into patients with Pelizaeus–Merbacher disease required administration of immunosuppressive drugs to limit potential rejection [49]. Similarly, transplantation of human embryonic stem cell (hESC)-OPCs into individuals with spinal cord injuries also was performed in conjunction with administration of immunosuppressive drugs [108,109]. Studies by Palmer and colleagues [110,111] have shown an important role for components of the innate immune response including NK cells in recognizing and rejecting MHC-mismatched NPCs following transplantation into the brains of mice. Similarly, we have demonstrated that engraftment of allogeneic NPCs into spinal cords of JHMV-infected mice results in rejection mediated, in part, by both T-lymphocytes as well as NK cells [112,113]. NPCs respond to both IFN-γ as well as viral infection in terms of expressing MHC class I and II as well as retinoic acid early precursor transcript-1 that allow for T-lymphocyte and NK recognition, respectively [112–114]. Collectively, these findings highlight that NPCs are recognized by cellular components of both the innate and adaptive immune response, indicating administration of immunosuppressive drugs must be considered in order to promote long-term survival and function.

Although syngeneic and allogeneic transplantation of mouse NPCs has generated valuable insights into mechanisms by which NPCs can function in an inflammatory CNS environment, another important research objective is to assess the therapeutic efficacy of engrafting human NPC or OPC-derived cells into JHMV-infected mice. We have previously transplanted high-purity predifferentiated human OPCs in mice undergoing JHMV-induced demyelination that resulted in limited clinical recovery [115]. Engrafted cells were rejected within 2 weeks post-transplantation even in the presence of immunosuppressive drugs targeting activated T-lymphocytes. Histologically, this resulted in only a slight increase in remyelination near the transplant site compared with HBSS transplanted mice [115]. This is in contrast to earlier studies using hESC-derived OPCs in a model of spinal cord injury in rat, where enhanced remyelination and improved motor function were observed following transplantation [116].

Alternatively, human NPCs have previously shown to exert neuroprotective effects in mouse and nonhuman primate models of EAE, suggesting they possess broader plasticity and function *in vivo* [117,118]. Indeed, we have recently demonstrated that engraftment of hESC-derived NPCs (hNPCs) into JHMV-infected mice with established demyelination resulted in clinical and histological improvement out to 6 months post-transplant although transplanted cells were rejected by day 8 following injection (Figure 3A & 3B) [119]. In contrast to mouse NPCs, the hNPCs neither migrated extensively from the site of injection nor appeared to differentiate into a neural lineage. hNPC-mediated recovery was associated with increased remyelination, but given that hNPCs were rejected within a relatively short period following injection it is unlikely these cells were directly contributing to remyelination [119]. Further, we do not believe remyelination was the result of acute inflammatory-mediated rejection as remyelinated axons were distributed both rostral and caudal to the implantation site rather than localized to the region of cell delivery. Mice transplanted with hNPCs showed reduced infiltration of CD4+ and CD8+ effector T cells into the spinal cords compared with transplant controls, while total numbers of CD4+CD25+FoxP3 regulatory T cells (Tregs) within the spinal cords were elevated (Figure 3C) [119]. Depletion of Tregs in hNPC-transplanted mice via anti-CD25 treatment inhibited the therapeutic benefits, highlighting the potential importance of these cells in contributing to hNPC-mediated recovery (Figure 3D). Cultured hNPCs secreted TGF-β1 and TGF-β2 compared with their undifferentiated hESC counterparts [119]. The roles of TGF- in impacting Treg maintenance and homeostasis are well established as previous work has shown that they promote FoxP3 expression in the peripheral Treg compartment, influencing their frequency and suppressive activity [120]. Therefore, one potential mechanism resulting in the enhanced recovery of these mice is via the immunomodulatory nature of the hNPCs, whereby expression of the anti-inflammatory cytokines TGF-β1 and TGF-β2 act in paracrine manner, influencing the local inflammatory environment within the spinal cord to promote accumulation of Tregs. An important role for Tregs during both acute and chronic JHMV infection has recently been demonstrated. IL-10-expressing virus-specific Tregs dampen proliferation of virus- specific effector CD4+ T cells and depletion of Tregs increases mortality, suggesting that during acute JHMV infection, Tregs limit immunopathological disease without negatively impacting viral clearance [121]. In addition, studies from Trandem *et al*. [122] have shown that adoptive transfer of Tregs to JHMV-infected mice attenuates clinical disease severity by dampening neuroinflammation and subsequently demyelination. These findings demonstrate the therapeutic potential of hNPCs in promoting sustained recovery through both promoting remyelination while limiting ongoing demyelination through muting neuroinflammation.

Expert commentary & five-year view

Mice infected with neurotrophic variants of MHV result in persistent infection that leads to chronic demyelination promoted by virus-specific and nonspecific T cells and macrophages. The histopathological similarities between MHV-infected mice with chronic neurological disease and MS make it an attractive model to understand the functions of NPCs in an inflammatory environment. In this review, we highlight recent advances regarding the use of both mouse and human NPCs in ameliorating the severity of neuroinflammation and

demyelination within the context of a viral model of the human demyelinating MS. This article has also provided an overview on the potential of NPCs in promoting remyelination following transplantation. Transplanted mouse NPCs physically bind and remyelinate axons resulting in increased axonal integrity. In contrast, remyelination observed following hNPC transplantation must occur by resident oligodendroglia as hNPCs are rapidly rejected below the level of detection. Furthermore, these studies reveal clear differences based upon the cell type used for therapeutic intervention: while transplantation of both mouse and human NPCs led to remyelination, only treatment with human NPCs resulted in a dramatic reduction in neuroinflammation accompanied by an overall improvement in clinical disease compared with mouse NPC transplantation. These findings demonstrate both the immunomodulatory nature and therapeutic potential of hNPCs. Importantly, studies derived from our viral model of MS illustrate that survival of transplanted cells is not required for restoration of motor skills. These observations indicate that immune-mediated rejection of allogeneic NPCs may not be important and that administration of immunosuppressive drugs may not be necessary to promote long-term recovery. Future research characterizing soluble factors released from hNPCs that impact the remyelination and affecting neuroinflammation will greatly aid in defining the molecular and cellular underpinnings leading to recovery. Moreover, identification of such factors may preclude transplantation of hNPCs as it may be possible to deliver these molecules to promote CNS repair and clinical recovery. Future research will be required to confirm and extend these findings. Additional research will continue to characterize the therapeutic potential of NPCs derived from induced pluripotent stem cells. Advantages of this approach include eliminating ethical issues confronting studies utilizing ESCs, the possibility of limiting allorejection (if this remains important) as hNPCs would be MHC matched to the patient and eliminating the need for lifelong treatment with immunosuppressive drugs and increasing ease in culturing and differentiation. An important unmet clinical need for MS patients is an effective method to induce sustained remyelination and limit immune cell infiltration into the CNS, and this emphasizes the importance of further investigation into the therapeutic potential of hNPCs.

Acknowledgments

This work was supported by National Institutes of Health (NIH) grants R01 NS041249 and R01 NS074987 (TEL) as well as National Multiple Sclerosis Society grant RG 4925 (TEL); NIH grant R01 GM-41514 (MDC); California Institute for Regenerative Medicine (CIRM) grants RM1-01717, CL1-00502 (JFL) and TR3-05603 (JFL).

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Key issues

- Identify mechanisms by which transplantation neural precursor cells (NPCs) improve clinical outcome through immunomodulation and promoting remyelination in different preclinical animal models of multiple sclerosis.
- Continue to define mechanisms by which allogeneic NPCs are recognized as foreign; explore whether components of the innate and/or adaptive immune response participate in rejection.
- Employ different preclinical models to confirm whether rejection of allogeneic NPCs affects clinical improvement.
- Define the therapeutic benefit of iPSC-derived NPCs in affecting disease progression and remyelination.
- Continue to interrogate the most effective route of NPC delivery, that is, intravenous, intrathecal or intraspinal injection.
- Identify soluble factor(s) secreted by NPCs that influence both immune responses and glial biology, for example, oligodendrocyte precursor cells maturation.

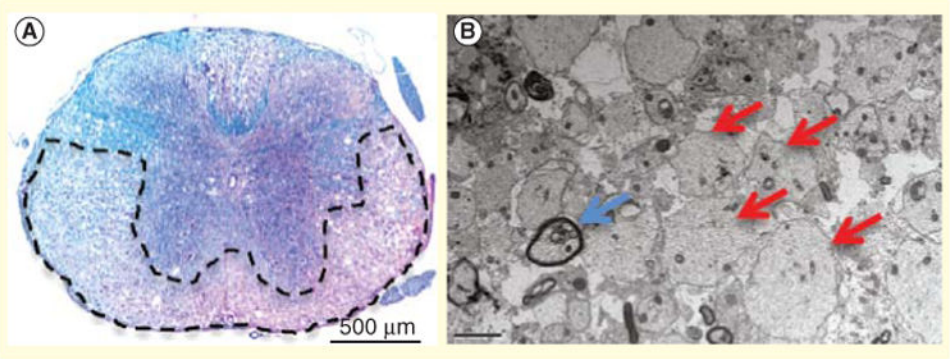

Figure 1. Histological characteristics of JHMV-induced neurologic disease

(A) A representative spinal cord was taken from an infected mouse at 5 weeks postinfection and stained with luxol fast blue to determine the extent of demyelination within the white matter tracts. Extensive demyelination is observed throughout the anterior and lateral regions of the white matter. **(B)** Electron micrograph images (1200×) showing demyelinated axons (red arrows) and remyelinated axons (blue arrow) at 5 weeks postinfection. Reproduced with permission from [119].

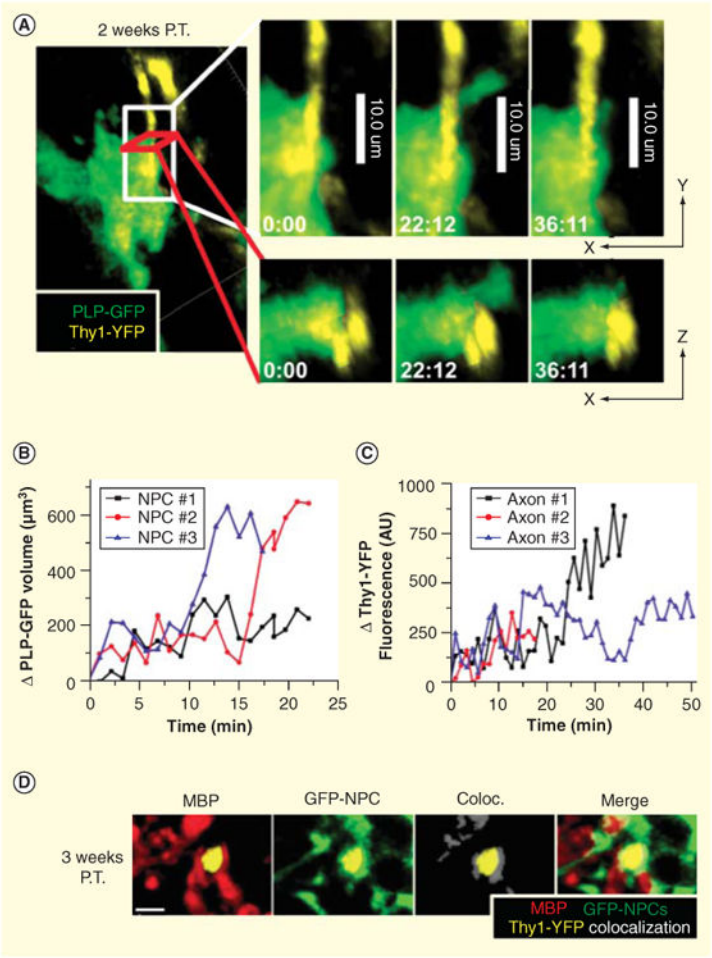

Figure 2. Axonal protection and remyelination transplantation of mouse neural precursor cells into JHMV-infected mice

Transplanted NPCs expressing cytoplasmic GFP driven by the myelin proteolipid protein promoter wrap around damaged axons. **(A)** Representative image showing colocalization between PLP-GFP (green) and damaged axons (yellow) in the JHMV-infected Thy1-YFP spinal cord 15 days post-transfer. Panels on the right show enlarged time lapse images of a PLP-GFP-positive cell wrapping around an axon (minutes:seconds). The top panels depict x–y sections, the lower panels x–z sections. **(B)** Analysis of the change in volume of PLP-GFP fluorescence of three different NPCs during wrapping as determined by a time lapse 3D reconstruction of the 2P data and the Imaris 'Surfaces' tool. **(C)** Analysis of the change in Thy1-YFP fluorescence intensity during PLP-GFP wrapping presented as arbitrary fluorescent units (AU). **(D)** Immunostaining in a transverse section of a JHMV-infected Thy1-YFP spinal cord 21 days after GFP-NPC transplantation. MBP (red), YFP^+ axons (yellow) and colocalization between overlapping GFP-NPC (green) and MBP fluorescence was determined using the Imaris colocalization tool (white). Merging of all three channels is shown on the right.

Scale bar = 4 μm.

NPC: Neural precursor cell.

Reproduced with permission from [107].

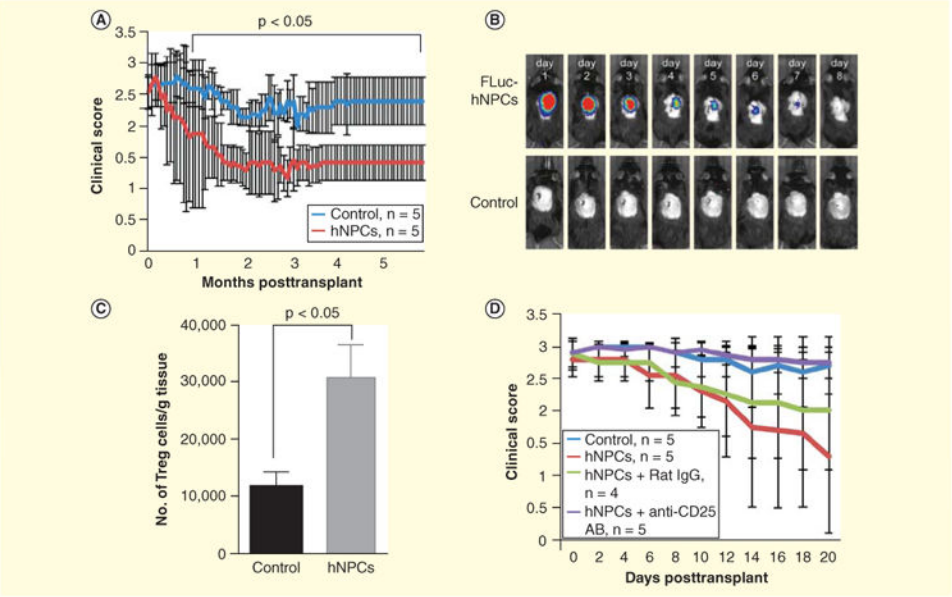

Figure 3. Intraspinal transplantation of hNPCs into JHMV-infected mice

(A) Improved ($p < 0.05$) clinical recovery in hNPC-transplanted JHMV-infected mice was sustained out to 168 days post-transplantation (p.t.) compared with infected mice treated with vehicle alone. **(B)** Daily IVIS® imaging of luciferase-labeled hNPCs revealed that following intraspinal transplantation, cells are reduced to below the level of detection by day 8 post-transplantation; representative mice are shown. IVIS imaging was performed on vehicle-transplanted mice as a control. **(C)** Quantification of Treg numbers in spinal cords of mice indicated a significant ($p < 0.05$) increase in numbers of Tregs in hNPC-transplanted mice versus controls between 8 and 10 days post-transplantation. Data are representative of three independent experiments with a minimum of three mice per group; data are presented as average ± SEM. Mann-Whitney t tests were used to determine the p-values. **(D)** hNPC-transplanted mice receiving anti-CD25 antibody (purple line) did not display recovery in motor skills compared with either hNPC-treated mice (red line), hNPC-treated mice receiving isotype-matched control antibody (green line) or vehicle control mice (blue line). Reproduced with permission from [119].