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Stem Cell Therapy for CNS Demyelinating Disease

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Abstract

Recent advances in cell-based therapies for demyelinating central nervous system (CNS) diseases have demonstrated the ability to restore damaged neuronal architecture and function. Demyelinated axons in patients with Multiple Sclerosis (MS) can spontaneously remyelinate over time; however, the rate and extent to which remyelination occurs is inadequate for complete recovery. Previous attempts aimed at regenerating myelin-forming cells have been successful, but nonetheless limited by the multifocal nature of the lesions and the inability to produce large numbers of myelin producing cells in culture. Stem cell based therapy can overcome these limitations to some extent and may prove useful in the future treatment of demyelinating diseases.

Introduction

In the CNS, myelin is produced by oligodendrocytes, and acts to insulate axons thus allowing rapid conduction of electrical signals and efficient communication between neurons. In addition, recent research has assigned another role for myelin as a determinant of axonal ion channel localization, which is also disrupted and contributes to loss of conduction in demyelinating disorders [1, 2]. MS represents the most common demyelinating disease of the CNS and is defined as a chronic inflammatory process resulting in eventual axonal loss from recurrent episodes of immune mediated attacks on myelin [3]. Clinically, most patients with MS have an initial relapsing and remitting course for the first 5-15 years followed by a secondary progressive course, which ultimately results in irreversible neuronal injury. In minority of cases, MS presents as a primary progressive type which sometimes can be fulminant [4]. The pathology associated with recurring episodes of MS is characterized by the presence of multifocal inflammatory infiltrates resulting in patchy demyelination or plaques, axonal loss, and astroglial scarring [4]. Initially, spontaneous remyelination of axons by endogenous oligodendrocyte progenitor cells (OPC) occurs, enabling neuronal conduction of action potentials to resume [5]. Unfortunately, the process of spontaneous remyelination fails over time as a result of the multifocal, chronic remitting and relapsing nature of the disease [6]. Currently, available treatments include immunosuppressing and immunomudulating agents which act only to reduce the rate of disease progression and have minimal effect on regeneration or repair.

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Remyelination in multiple sclerosis

In order to study the process of demyelination and remyelination, a number of experimental animal models have been developed. These include: 1) Toxic demyelination, which requires the injection of toxins such as lysolecithin into the white matter leading to selective myelin loss. 2) Inflammatory demyelination, such as experimental autoimmune encephalomyelitis (EAE), which is induced by injecting animals with myelin proteins to which the animal mounts an immune response. 3) Viral demyelination, which results from infection with Theiler's murine encephalomyelitis (TMEV), Murine hepatitis virus (MHV) or Semiliki Virus (SV). 4) Transgenic models with genetic defects leading to inadequate myelin formation. Using these models, the mechanisms of myelin disease and repair can be elucidated.

The CNS is capable of remyelinating axons after demyelination has occurred. However, the newly formed myelin sheath is usually thinner and shorter compared to the previous myelin sheath [7]. In EAE experimental mouse models, spontaneous remyelination during the early stages of the disease process occurs in response to the immune mediated attack on myelin [8]. Similar findings have been observed in MS patients [9-11]. These findings are consistent with the clinical improvements seen following acute exacerbations of EAE or MS. Furthermore, clinical studies in patients with the recent onset of MS have demonstrated a higher density of oligodendrocytes at the lesion sites [12-13]. In contrast, patients with chronic MS demonstrate oligodendrocyte loss and little or no remyelination [13]. These findings suggest that although initial attempts at remyelination by endogenous oligodendrocytes are successful, chronic disease may overwhelm these repair mechanisms resulting in permanent loss of neurons.

It has been shown in experimental animal models that mature oligodendrocytes, whether transplanted [14] or endogenous, [15] do not have the ability to remyelinate demyelinated regions of the CNS. These results suggest that remyelination is mediated by progenitor cells that must migrate to the affected site and differentiate into mature myelin producing cells. In fact, it has been shown that the cells responsible for the remyelination process are not the existing postmitotic oligodendrocytes found throughout the white matter but, instead, the OPC population found in the subventricular zone [14-15].

Recently, a review by Franklin [6] outlined pitfalls that may be encountered along the pathway of the remyelination process, as the OPC must migrate to the area of demyelination and subsequently differentiate into myelin producing cells and ensheath axons. These include: 1) Failure of OPC recruitment to the demyelinated region. Growth factors such as PDGF and FGF were shown to play critical roles in OPC recruitment in viral [16] and toxic [17] animal models of demyelination. However, little is known about the endogenous expression of growth factors and their role in recruiting the progenitor cells. 2) Depletion of OPC. Previous data have shown that repeated cycles of demyelination and remyelination throughout the course of the disease result in depletion of OPC stores thus preventing recruitment of myelin producing cells to the lesion site over time [18, 19]. 3) Inhibition of remyelination as a result of an imbalance between factors promoting inflammation and those promoting remyelination. Pathology data suggest that remyelination is most effective in

acute lesions where the inflammatory response is most active [20]. Studies using a toxic demyelination animal model have shown that cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), play critical roles in myelin repair [21, 22]. Furthermore, it was shown that human neural stem cells which express chemokine receptor 4 (CXCR4) migrate and bind to the inflammatory chemoattractant stromal cell-derived factor 1α (SDF-1α), produced by astrocytes and endothelial cells in the infracted regions of the brain [23]. These data imply that factors involved in inflammation may act to recruit OPC and neural stem cells to the site of injury, suggesting that anti-inflammatory processes and/or premature decline in the inflammatory response may terminate recruitment of critical components before myelin recovery is completed. 4) Failure of OPC to gain access to the demyelinated site as a result of a scar formation or astrogliosis. Reactive astrocytes produce a large number of growth factors that promote OPC proliferation and differentiation [24]. However, chronic demyelination may lead to the formation of glial scarring thus preventing OPC entry to the lesion site. 5) Failure of OPC differentiation. A number of factors have been shown to promote or inhibit OPC differentiation such as Insulin-like growth factor 1 (IGF1) [25] or neuregulin [26]. Neuregulins are ligands homologous to the EGF family of growth factors. They are produced by neurons and glia, and act to promote oligodendrocyte proliferation, survival, and differentiation depending on the stage of development.

Experiments by Chang and collaborators [27] suggest that OPC can differentiate into premyelinating oligodendrocytes in chronic lesions of MS patients with clinical disease lasting 1-15 years. However, these cells may not be able to receive appropriate signals to produce myelin as a result of abnormal molecular composition of demyelinated axons, such as expression of PSA-NCAM. PSA-NCAM is a neural adhesion molecule that is expressed on axons during development and acts to inhibit myelination. Evidence suggests that PSA-NCAM is re-expressed on demyelinated axons and may act to inhibit OPC attachment to axons [27]. Therefore, even after the OPC have been recruited and differentiated into mature oligodendrocytes, the challenge then remains for these cells to make appropriate contacts and to receive accurate signals from the demyelinated axons in order to produce myelin.

Finally, an intriguing yet controversial role in the remyelination process has been played by astrocytes. Astrocytes produce a number of growth factors that have been shown to regulate OPC proliferation and differentiation. It was recently demonstrated that astrocyte-expressed neuregulin was markedly reduced in active MS lesions and that lack of this growth factor may contribute to the abnormal development of oligodendrocytes [28]. In addition, it was shown that astrocytes in demyelinated lesions express Jagged-1, which acts through Notch-1 receptors to inhibit oligodendrocyte differentiation [29]. In contrast, astrocytes in remyelinated lesion did not express Jagged-1. In summary, these findings reveal a complex array of factors and pathways that may be involved in the remyelination process and that although there is clearly an endogenous response to myelin and neuron damage in the central nervous system, it appears that the mechanisms activated to combat demyelination are insufficient to promote long term recovery and regeneration to a previous state.

Stimulation of endogenous remyelination

Endogenous remyelination in the CNS has been recently attempted by using growth factors, such as NGF and IGF-1, in order to support endogenous OPC recruitment and activation. Villoslada and collaborators showed that administration of NGF delayed the onset of EAE by downregulating the production of interferon gamma by T cells, and by upregulating the production of interleukin 10 by glial cells in the inflammatory lesions [30]. However, while initially treatment of EAE animals with IGF-1 showed some beneficial effect in the acute phase of the disease, it was only minor in the chronic phase. Furthermore, a trial in which seven MS patients were treated with recombinant IGF-1 twice a day for 24 weeks did not show any clinical improvements or changes in MRI when compared to a 24 week baseline [31]. These findings imply that less than expected outcomes involving single growth factor treatments should not be surprising, since there are large numbers of temporally expressed and uniquely balanced growth factors required during the myelination and remyelination processes [32]. Therefore, future experimentation utilizing growth factor treatments will require balanced combinations of multiple factors. Currently, other growth factors being applied with some success in EAE and toxic demyelination animal models include neuregulin, LIF, FGF-2 and PDGF.

Another method for inducing endogenous remyelination relies on the generation ofimmunoglobulins (Ig) directed against myelin components. Rodriguez and Lennon demonstrated that remyelination of the CNS axons in a viral demyelination mouse model, was promoted by systemic injection of serum derived from donor mice hyperimmunized with homogenized spinal cord [33]. Although the mechanism by which these antibodies promote remyelination is not clear, it is thought to be an indirect effect influencing the immune response, such as protecting oligodendrocytes from complement, rather than a direct effect on any one component or cell type. Recently, it was shown that serum derived human monoclonal IgM could promote remyelination in the viral and toxic demyelination mouse models, suggesting a potential therapy to enhance myelin repair following CNS injury and disease [34, 35]. Furthermore, Stangel and collaborators conducted a double blind, placebo controlled pilot study to evaluate the effect of immunoglobulin treatment in patients with MS [36]. Unfortunately, their results do not support a role for immunoglobulin treatment in the remyelination of MS. The patients treated in this study were chronic MS patients, supporting the notion that if immunoglubulins indeed act to mediate the immune response rather than to directly influence oligodendrocytes, treatment of MS patients with immunoglobulins would have to be continuous even before a relapse would occur. Therefore, immunoglubulins may still hold as a potential therapeutic for MS patients but much needs to be elucidated.

Neuronal replacement therapies may be possible by influencing endogenous neural precursors to differentiate. Recent findings described by Magavi and collaborators [37] demonstrated that endogenous neural progenitor cells can be manipulated *in vivo* to differentiate into cortical projection neurons. These neurons subsequently form appropriate connections in the regions of the cortex that otherwise do not undergo neurogenesis. Protein or gene delivery such as infusion of recombinant proteins [38] or adenoviral vectors [39] can

also act to stimulate endogenous neural progenitor cells. These methodologies are currently being investigated with promising preliminary findings.

Transplantation of myelin forming cells

Over the last four decades, a number of approaches has been implemented in attemptingto transplant myelin-forming cells into animal models of MS. However, these methods havebeen hindered by many factors such as the inability of myelin producing cells to grow in largenumbers *in vitro*. For example, experiments in which the clonal development of oligodendrocytes was studied by culturing individual oligodendrocyte type-2 astrocyte (O-2A) progenitor cells showed that oligodendrocytes differentiated after a fixed number of cell divisions [40]. Another limitation includes the inability to migrate and remyelinate axons outside the transplantation site *in vivo*. Experiments involving the use of gliotoxin-induced demyelination, X-irradiation, and glial cell transplantation, showed that the recruitment of remyelinating cells takes place over a very limited area [41].

Transplantation of Schwann cells in order to repair demyelinated neurons in the CNSinitially showed great promise, since these cells might have avoided the immune attack initiallygenerated against oligodendrocytes while acting to remyelinate axons in MS [42]. Schwann cellsare readily accessible and can be expanded *in vitro*, however, phase I clinical trials performed onMS patients showed that autologous Schwann cells that had been transplanted into single demyelinating lesions failed to survive five months after transplantation (http://www.myelin.org/06232003.htm).

Finally, a new source for myelin has been recently found in olfactory ensheathing cells (OEC) [43]. Human OEC were isolated, expanded, and shown to remyelinate demyelinated rat spinal cords [44]. This may prove to be another source of autologous cells that can be harnessed for remyelination.

Stem cell based therapy for the treatment of multiple sclerosis

In recent years, the use of stem cell based therapy has become the focus of many attempts to overcome the issues of generating unlimited numbers of myelin producing cells and the ability to access multiple areas of demyelination as observed in MS. In considering a cell source, stem cells fulfill the requirement of differentiating to a specific fate, depending on the environmental cues. Stem cells are pluripotent and proliferative, thus allowing for an unlimited production of any cell type. The two most common sources have been embryonic and adult stem cells. Embryonic stem cells are pluripotent cells that are found in the inner cell mass of the developing embryo and give rise to all cell types including hematopoetic precursors, heart and skeletal muscle, endothelium, and neural cells [45]. Human embryonic stem (ES) cells can be propagated and maintained in culture almost indefinitely [46]. Furthermore, it has been demonstrated that these cells can differentiate to neural lineages under the influence of certain growth factors and mitogens [47]. Transplantation experiments in a rat model of myelin disease showed that ES cell-derived precursors could interact with host neurons and efficiently myelinate axons in the brain and spinal cord [48]. These experiments have yielded promising results, however, a noted complication of the ES cell transplantation has been the formation of malignant teratomas as observed after ES cells

were transplanted into mouse hippocampus [49]. Experiments by Zhang and collaborators [50] demonstrated that selective growth conditions can give rise to specific precursors which could be successfully transplanted without the production of malignant teratomas. These data suggest that cell type specific neural precursor rather than pluripotent ES cell population might be better suited for transplantation and subsequent differentiation in the neuronal environment.

Adult stem cells were initially identified in the hematopoetic system, gut, and skin, where cell turnover occurs at a high rate throughout life. Recently, stem cells have been identified in brains of adult rodents and humans [51, 52]. Specifically, they are localized in the subgranular zone in the dentate gyrus of the hippocampus and the subventricular zone (SVZ). Like other stem cells, adult stem cells can divide asymmetrically producing a daughter cell and a more committed progenitor. *In vitro* experimentation has shown that adult stem cells can expand and adopt a neuronal fate under the influence of brain-derived neurotrophic factor (BDNF) or astrocytic fate in the presence of bone-morphogenetic proteins (BMP) [53]. Furthermore, *in vivo* experimentation has shown that these cells have the capacity to migrate to and differentiate into cells required to repair specific damaged tissue. For example, Pluchino and collaborators showed that adult stem cells that were injected either intravenously or intracerebroventricularly into mice with the EAE, could specifically enter demyelinated regions within the CNS and could differentiate into mature cells producing myelin [54]. As a result, the functional impairments caused by EAE were reversed almost completely. Taken together, these findings suggest that adult neural stem cells may prove to be a valuable source for regeneration of the components of the CNS; however much needs to be elucidated on differentiation and cellular integration of these cells.

Bone marrow stem cells (BMSC) represent a common source of stem cells as well as potential candidate for utilization in demyelinating diseases since they can renew throughout adult life. In addition, adult BMSC can transdifferentiate into a number of cells types such as muscle, skin, liver, lung, and neuronal cells in mice with the ability to regenerate myocardium, hepatocytes, skin, and gastrointestinal epithelium in humans. Recent evidence suggests that adult human BMSC can be expanded *in vitro* and subsequently transplanted into the human brain with resultant neuron formation in the hippocampus and cerebral cortex [55]. In addition, the functionality of transplanted BMSC was demonstrated in rodent studies where implantation or intravenous infusion led to remyelination in the spinal cord [56] and functional improvements in cerebral ischemia models [57]. Based on these animal model data, about 250 patients have received BMSC transplants for MS. Phase I and Phase II trials have been promising based on the MRI data and clinical improvements [58]. Phase III trials are currently underway and hold much promise.

The mechanism by which BMSC may contribute to neuron formation is not clear. One possibility includes transdifferentiation, the direct conversion of transplanted BMSC to neurons. Another mechanism may rely on fusion of BMSC with the ES cells. Recently, Alvarez-Dolado and collaborators showed that BMSC could fuse with Purkinje neurons in the brain, hepatocytes in the liver, and cardiac muscle cells in the heart [59]. These findings support a mechanism whereby cell fusion may allow the formation or replacement of

specific cell types. Taken together, these findings support BMSC as an exciting candidate for replacing damaged or dead cells in the CNS. However, the extent of migration, differentiation, and functionality *in vivo* must be investigated further.

A key issue in stem cell therapy, especially for the multifocal nature of MS lesions, is mode of delivery. Direct transplantation of stem cells for certain unifocal lesions such as those observed in ischemia, Parkinson's disease, or spinal cord injuries may be expected to promote regeneration of diseased tissue when grafted into those sites. The challenge for treating the multifocal lesions of MS is to deliver stem cells to multiple damaged sites that cannot all be reached by direct intralesional transplantation. Recent experiments have shown the ability to overcome this limitation by injecting stem cells intravenously, directly into the blood stream, or intracerebroventricularly, into the cerebrospinal fluid (CSF). In a series of elegant experiments, Pluchino and collaborators [54] demonstrated that intravenously or intracerebroventricularly delivered mouse adult neural stem cells could promote functional and anatomical recovery of myelin sheaths in the EAE mice. They postulate that the injected stem cells circulate within blood or CSF compartments until they reach an inflamed region of the brain parenchyma or spinal cord. The mode by which these cells then recognize damaged tissue can be mediated by the expression of a number of inflammatory molecules by the transplanted stem cells. For example, different migration patterns of transplanted neural stem cells into animals with experimental hippocampal lesions might be due to differences in integrin signaling pathways [60]. Integrins are expressed on neural precursor cells and have been shown to regulate migration of neural crest cells during development [61]. Integrin expressing stem cells in the circulation can associate with integrin receptors found on activated endothelial cells surrounding damaged tissue. Once bound, the transplanted stem cells may be further influenced by chemokines or cytokines and subsequently attracted to the lesion sites [23, 62].

Stem cell therapy for other neurodegenerative diseases

Once stem cells have reached their targets, the goal is to differentiate and restore lost function. Most data suggest that the local environment to which stem cells migrate will dictate their fate. In the EAE and chemical model of demyelination, stem cells have shown a differentiation pattern specific for oligodendrocytes after they have been injected intravenously or transplanted into the rat spinal cord [54, 63]. Although remyelination has been observed, little is known about the mechanisms responsible for lineage specific differentiation.

Stem cell therapy has had some success in other neurological disorders such as Parkinson's disease, Huntington's disease, and stroke. Parkinson's disease is due to the degeneration of dopaminergic neurons in the substantia nigra resulting in tremor, bradykinesia and rigidity. Initial clinical trials using transplanted fetal dopaminergic neurons did show promising results, however, these studies were hindered by ethical issues. A new strategy for treating Parkinson's disease with stem cells was implemented, in which a large number of dopaminergic neurons were generated *in vitro* from ES cells or neural stem cells. These stem cells were induced into dopaminergic neurons in culture, purified via reporter selection and FACS analysis, and subsequently implanted into the rat striatum [64]. Recently, it was

shown that dopaminergic neurons generated from monkey ES cells that were transplanted into primate models of Parkinson's disease could diminish symptoms observed in this neurodegenerative disorder. These findings describe the first accounts of successful treatment of Parkinson's disease in primates and hold great promise for future treatment methods.

Huntington's disease is an inherited, autosomal dominant disorder in which patients exhibit chorea and dementia. Transplantation of fetal striatum into animal models of Huntington's disease has shown to reverse behavioral defects [65]. Furthermore, primate studies have shown that fetal grafts may extend to cognitive function improvement as well [66]. More recently, human fetal stem cells were shown to promote behavioral and anatomical recovery when transplanted in a rodent model of Huntington's disease [67].

Stroke is a common cause of neuronal death and it is becoming evident that neurogensis takes place following an ischemic event, in an attempt to replace lost cells. Transplantation of neural stem cells in the rat models of stroke improves motor and cognitive function [68]. Furthermore, neural transplantation in patients with stroke is feasible based on PET scan data which showed improvements in the implantation sites [69]. Interestingly, BMSC were shown to be recruited to ischemic regions of the brain following a stroke in animal models [70]. Furthermore, recent findings show that injection of BMSC in animal models of stroke can lead to functional improvements [71]. The exact mechanism of this process is unknown and is currently under investigation.

Conclusion

Remyelination in MS patients is inadequate to repair demyelinated axons. Therefore, numerous attempts to accomplish efficient remyelination in the MS have been tried. Growth factors, such as IGF-1, did not lead to any clinical improvement (Table. I), which may be due to other growth factors that may be required simultaneously or some other sequence of factors, in addition to IGF-1, that need to be present at specified time points for efficient myelination to take place. Patients treated with the intravenous immunoglobulins also failed to respond to treatment but this could be attributed to the fact that the trial included patients with stable MS while immunomodulation might be more beneficial before a relapse or even administered continuously (Table. I). Furthermore, the autologous transplantation of Schwann cells in MS patient was attempted (Table. I). These cells are a favorable source since they are easily accessible via biopsy, could be expanded *in vitro*, have a low risk of rejection, and could potentially evade the immune system. Unfortunately, this trial was terminated since no clinical improvement was noted after transplantation.

The use of stem cell therapy for the regeneration of damaged CNS components is promising in the light of recent advances in animal model studies and to some degree in humans (Table. I). The utilization of the ES cells poses serious ethical dilemmas; however, the potential of adult stem cells and bone-marrow stem cells is enormous and could offer alternatives for stem cell based therapies.

It is now evident and accepted that the renewable cell pool exists in the adult CNS. However, many unanswered questions remain: 1) what is the optimal mode of stem cell delivery, and is it disease specific? 2) how can isolated stem cells be transplanted with a balanced potential for multiple lineages but low potential for transformation into malignant cells? 3) when and where should stem cells be transplanted during the course of a disease? 4) what factors are required for lineage specific differentiation and migration? 5) how can axonal damage be minimized until stem cells initiate their effects? 6) finally, how might gene therapy help to influence stem cell fate prior to transplantation? The ultimate goal of future studies will be to effectively restore the anatomical and functional components of the CNS affected in demyelinating disorders with complete physical recovery, in a manner free of negative short and long-term side effects.

References and Recommended Reading

* Of importance

** Of major importance

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Table I

Summary of MS Clinical Trials

Growth Factor	
IGF-1	Frank *et al*., 2002
Immunoglobulin	
IVIg	Stangel *et al*., 2000, Noseworthy et al., 2000, 2001
Myelin-Forming Cell Transplants	
Schwann Cells	http://www.myelin.org/06232003.htm
Stem Cell Transplants	
Bone Marrow	Blanco *et al*., 2005