Stem cell transplantation in multiple sclerosis: current status and future prospects

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Abstract | This article provides an overview of the current knowledge relating to the potential use of transplanted stem cells in the treatment of patients with multiple sclerosis (MS). Two types of stem cells, CNS-derived neural stem/precursor cells (NPCs) and bone marrow-derived mesenchymal stem cells (MSCs) are considered to provide reproducible and robust therapeutic effects when intravenously or intrathecally injected into both rodents and primates with experimental autoimmune encephalomyelitis. Furthermore, preliminary safety data concerning the use of intrathecally injected autologous MSCs in patients with progressive MS are available. We discuss how the data gathered to date challenge the narrow view that the therapeutic effects of NPCs and MSCs observed in the treatment of MS are accomplished solely by cell replacement. Both types of stem cell, when transplanted systemically, might instead influence disease outcome by releasing a plethora of factors that are immunomodulatory or neuroprotective, thereby directly or indirectly influencing the regenerative properties of intrinsic CNS stem/precursor cells.

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Introduction

The potential of stem cell-based therapies to revolutionize the treatment of neurological disorders is an exciting prospect for modern medicine. Currently, the multiple sclerosis (MS) field is rife with stem cell-related hype, misunderstanding and ambiguity among scientists, clinicians and the public. Consequently, we urgently need a consensus among professionals, based on existing solid scientific and clinical evidence, that will assist patients in distinguishing between fanciful and realistic therapies.

The UK and US National MS Societies, with the support of national MS Societies from Italy, France, Canada and Australia, organized a meeting in London, UK on 19 May 2009 with the sole aim of producing a consensus statement (Box 1) on the use of stem cell therapies in MS. 29 stem cell and MS experts, along with 17 representatives from the MS societies (Box 2), gathered together and discussed the potential role of stem cell therapies in the treatment of MS. Debates centered on expectations of stem cell therapies, based on existing knowledge, and whether certain types of stem cell therapy might now be progressed towards clinical trials. Extensive clinical and experimental data exist on hematopoietic stem cell therapy in MS, which is a rescue therapy for the most aggressive forms of the disease and is aimed at modulating or 'resetting' the immune system.[1–4] This Review, however, focuses on transplantation of mesenchymal stem cells (MSCs) and various stem and precursor cell populations of neural origin as potential stem cell treatments in MS.

Competing interests
The authors declare no competing interests.

The current status of MS therapy

MS is an immune-mediated disease of unknown etiology (Box 3). There are two generally accepted strategies for treating MS: preventing CNS damage indirectly through immunomodulatory interventions, and repairing CNS damage by promoting remyelination. These approaches have the potential to address the unmet need of providing neuroprotection, either by reducing inflammation that causes axon damage in the acute and possibly chronic lesion (immunomodulation), or by restoring functionality of the myelin sheath on which axonal health depends (remyelination).

Currently approved disease-modifying drugs for MS include interferon β, glatiramer acetate, natalizumab and mitoxantrone, but these treatments are only effective in relapsing forms of MS—both relapsing–remitting (RRMS) and relapsing–progressive—since they act primarily by suppressing the immune response. These drugs might also have a limited inhibitory effect on neurodegeneration or disease progression, but no clear repair-promoting activity has yet been associated with their use.[5] The next generation of MS disease-modifying therapies, such as alemtuzumab, rituximab, cladribine, fingolimod and laquinimod,[6,7] are likely to be more efficacious than the currently available treatments for relapsing forms of MS, particularly RRMS. However, these therapies will not benefit the large numbers of patients in the progressive phase of MS, who have advanced disability and represent the main social burden of this disease. Similarly, therapies that prevent demyelination and neuronal damage do not directly address axon pathology,[8,9] the mechanism of which might be quite distinct from the neuronal apoptosis seen in diseases such as stroke and dementia. Treatment

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Key points

- Therapies based on neural stem/precursor cells (NPCs) or mesenchymal stem cells (MSCs) might limit neuronal damage in patients with multiple sclerosis (MS) by directly or indirectly promoting neuroprotection via remyelination and immunomodulation
- Intravenously or intrathecally delivered NPCs have immunomodulatory effects in both the CNS and the periphery; NPCs probably contribute directly to remyelination when delivered into areas of demyelination
- Intravenously delivered MSCs have peripheral immunomodulatory effects and might indirectly influence remyelination
- Exploratory trials using MSCs and NPCs to treat early secondary progressive MS that is refractory to conventional therapy should now be considered
- The International Society for Stem Cell Research guidelines should be strictly followed, and patients should be discouraged from approaching non-regulated 'stem cell clinics'
- All trials should be prospectively registered, and sharing of methodologies and data should be encouraged

Box 1 | The STEMS Consensus Group consensus points

- The main objective of multiple sclerosis (MS) therapy is neuroprotection. Two processes—immunomodulation and remyelination—could potentially provide neuroprotection in MS, and neural stem/precursor cells (NPCs) and mesenchymal stem cells (MSCs) could be integral to both.
- NPCs are immunomodulatory and indirectly support remyelination when given intravenously or intrathecally. They directly contribute to remyelination when injected into or near areas of demyelination.
- Intrathecal injection of NPCs is preferred to avoid unwanted peripheral side effects; dose-escalating clinical trials are recommended.
- Fetal tissue is the only clinically approved source of NPCs at present. In the future, NPCs derived from human embryonic stem cells or induced pluripotent stem cells might become available. Preclinical studies are required to establish the therapeutic value of autologous NPC sources.
- MSCs have powerful immunomodulatory effects when administered systemically.
- Intravenous administration of MSCs is the preferred route for transplantation of these cells owing to their peripheral immunomodulatory effects. Intrathecal injection of MSCs might provide neuroprotective benefits to patients with MS and should be further investigated.
- Exploratory trials using MSCs and NPCs to treat patients with early secondary progressive MS that is refractory to conventional therapy should now be considered.
- Clinical trials should adhere to both the International Society for Cellular Therapy and International Society for Stem Cell Research guidelines.
- Information detailing the source and purity of the stem cells used in clinical trials should be freely available to the public and to the scientific community.
- Information regarding safety and ethical issues should be incorporated in the informed consent documentation.
- Patients with MS should be discouraged from approaching stem cell clinics that do not follow the recommendations outlined above.
- All trials should be prospectively registered and the investigators should be encouraged to share methodologies and data.

of the neurodegenerative component of MS, including remyelination failure and axonal and/or neuronal loss, is still far from being established.

The role of stem cells in MS therapy

Remyelination in MS can be a fairly extensive spontaneous process, mediated by a population of endogenous adult neural stem cells.[10–12] Unfortunately, this process is not sustained and remyelination often fails, resulting in chronic demyelination and progressive axonal atrophy.[13] Given that endogenous stem cell-mediated remyelination can occur in patients with MS, a strong sense prevails in the MS field, including within this consensus group, that promotion of endogenous remyelination could be a feasible and perhaps imminently available stem cell therapy for this disease. This optimism is based on an increasing understanding of the pathways that regulate endogenous stem cell-mediated remyelination, which are potentially amenable to pharmacological manipulation.[14,15] However, without meaning to understate the importance of this stem cell-based approach to the regenerative medicine of MS, this method of promoting remyelination is beyond the scope of this Review, as the focus of the consensus meeting was on stem cell transplantation. For a discussion of strategies for promoting endogenous remyelination, the reader is referred to two recent reviews.[12,16]

Exogenously administered stem cells could potentially contribute to immunomodulation and remyelination. A wealth of preclinical data suggests that both MSCs and neural stem/precursor cells (NPCs), on transplantation, exert multifaceted therapeutic effects, via mechanisms other than cell replacement, transdifferentiation or fusion.[17–32] These stem cells probably exert their principal neuroprotective effects by secreting a complex array of factors with immunomodulatory and neurotrophic properties that might influence CNS-confined inflammation and/or endogenous remyelination. These generic properties of somatic stem cells[33] could, in part, account for why cells with very low neural transdifferentiation capabilities *in vivo* might, nevertheless, help promote endogenous repair processes.[18,34] This bystander process is the basis of the concept of 'therapeutic plasticity' (Figure 1),[34] in which somatic stem cells adapt their fate and function to specific environmental needs arising from different pathological conditions.

Mesenchymal stem cells

The adult bone marrow contains a nonhematopoietic cell lineage that is capable of differentiating into osteoblasts, adipocytes and chondrocytes. These cells are currently termed MSCs, owing to their preferential capacity for differentiating into cells of the mesodermal lineage. MSCs constitute the stromal scaffold that provides the appropriate microenvironment for maturation and differentiation of blood-derived progenitor cells, possibly through the release of survival factors.[18,35] In addition to the bone marrow, MSCs also reside in adipose tissue and muscle, where they are termed adipose-derived stem cells and muscle-derived stem cells, respectively. The MSC niche is not completely characterized, but wherever MSC-like cells are present in the body they are most likely to be found in close contact with blood vessels and tissue-specific stromal cells such as pericytes. Under some experimental conditions, MSCs have been reported to transdifferentiate into cells from the two other germinal lineages—the ectoderm and the endoderm. Furthermore, owing to the relative simplicity

of their culture *in vitro,* and preclinical evidence demonstrating that intravenously infused MSCs improve the clinical course of mice with experimental autoimmune encephalomyelitis (EAE),[19] MSCs currently represent an ideal source of adult stem cells that are amenable to therapeutic development in degenerative and immune-mediated diseases, including MS.

Immunomodulatory properties

Data obtained from studies performed in EAE mice,[19–21] as well as in patients with acute graft versus host disease (GVHD),[36] suggest that MSCs can interact with cells of both the innate and adaptive immune systems and modulate their function. The mechanisms that sustain the immunomodulatory effect of MSCs *in vivo* are still under scrutiny, although several processes have been associated with MSC transplantation. For example, MSCs can arrest cell division,[37] induce T cell anergy, affect B lymphocyte proliferation and maturation,[38] and migrate to inflammatory areas under the guidance of both cell adhesion molecules and receptors for inflammatory chemokines.[18]

Reparative mechanisms

MSCs could promote survival of damaged tissues through secretion of large amounts of bioactive factors that inhibit scarring and apoptosis, as well as by stimulating angiogenesis, and mitosis of tissue-intrinsic stem or progenitor cells.[18] These cells might, therefore, be used to promote structural and functional repair of damaged tissues.[39–41]

MSCs can transdifferentiate into cells of the neuroectodermal lineage and, therefore, might also contribute to cell replacement in the injured CNS.[35,42–44] However, the methods used to promote neural cell differentiation *in vitro* and to assess the biology of the differentiated cells remain contentious.[18,44] The idea that MSCs can give rise to remyelinating cells when transplanted directly into areas of experimental demyelination is also debatable.[18,44,45] In our view, transplanted MSCs are unlikely to be viable therapeutic options for direct remyelination in the near future, but we cannot exclude the possibility that MSCs will indirectly promote endogenous oligodendrogenesis.[46]

Cell sources

MSCs can be isolated from bone marrow, skeletal muscle, adipose tissue, synovial membranes and other connective tissues of adults, as well as umbilical cord blood and placental products (Box 4). They can be identified by a combination of phenotypic markers and functional properties, but controversy still exists over the *in vivo* phenotype of MSCs. *Ex vivo*-expanded MSCs can be identified by flow cytometry, as these cells express CD73, CD90 and CD105 cell-surface markers, but do not express the hematopoietic markers CD14, CD34, CD45 or MHC class II.

Despite the fact that the yield of MSCs obtained from bone marrow and expanded *in vitro* never exceeds $1–3 \times 10^6$ cells per kg, bone marrow is the preferred source

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Box 3 | Multiple sclerosis

Multiple sclerosis (MS) is an acquired inflammatory and neurodegenerative immune-mediated disorder of the CNS, characterized by inflammation, demyelination and axonal degeneration.[94] This condition is a complex multifactorial disorder, in which both environmental and genetic factors are thought to contribute to the underlying etiology.[6,95–99] In particular, CNS autoreactive T cells are thought to interact with CNS myelin antigens and initiate a proinflammatory cascade within the brain that results in either target-directed immune damage or bystander damage. Approximately 80–85% of all patients present with a form of the disease known as relapsing–remitting MS (RRMS), which is characterized by unpredictable, acute episodes of neurological dysfunction, followed by variable recovery and periods of clinical stability. The remaining 10–15% of patients develop a progressive form of MS from disease onset, which is called primary progressive MS. Within 10 years of diagnosis, >50% of patients with RRMS develop sustained neurological deterioration with or without further relapses. This form of the disease is called secondary progressive MS.

of MSCs for transplantation purposes. Although such a dose has been successful in controlling severe acute GVHD,[36] this titer does not seem to be sufficiently large to be used successfully in MS, since the number of cells infused in EAE mice is usually 30–40-fold higher. Therefore, clinical studies based on infusing $1–3 \times 10^6$ cells per kg might fail. Alternative sources of MSC that provide a higher yield of MSCs, such as amniotic fluid-derived cells, should be considered in the future.[47]

Route of administration

Ex vivo-expanded allogeneic MSCs have been administered by intravascular infusion in several phase I studies,[36,48–50] and to date no adverse events have been observed either during or after infusion. Furthermore, no evidence of ectopic tissue formation has been observed after MSC infusion. By extrapolation from observations in animal models, MSCs delivered into humans by intravascular infusion are unlikely to remain in the circulation for more than 1 h.

MSCs exert immunomodulatory effects both in the periphery and in the CNS when given either intravenously or intrathecally. Intravenous administration of MSCs is preferred, because this method of delivery has been established to be safe in leukaemia patients with severe GVHD.[36] Preliminary evidence also suggests that MSCs given intravenously to EAE mice might indirectly influence remyelination via peripheral immunomodulation.[19,20] Intraparenchymal MSC injection should be avoided, since no convincing preclinical data are available to indicate that this method of delivery is safe or has any associated reparative effects.[45]

Patient selection

Two trials have used a single intrathecal injection to administer autologous MSCs to patients with MS.[51,52] Although the results are preliminary and long-term follow-up is lacking, the findings from the two studies suggest that this procedure is safe. In light of these encouraging safety data, proof-of-principle studies should be conducted in patients with early secondary progressive MS (SPMS) who are refractory to conventional therapy. Patients with primary progressive MS with ongoing CNS-confined inflammatory activity—as indicated by the presence of gadolinium-enhancing MRI lesions and positive cerebrospinal fluid oligoclonal banding—have also been suggested as suitable candidates for receiving MSC therapy.[53] Repeated MSC injections, however, should be avoided until the long-term safety of single intrathecal MSC injections is clearly established.

Neural stem/precursor cells

The adult CNS contains a heterogeneous population of mitotically active cells that have complex patterns of gene expression.[33,54] These CNS stem cells have a virtually unlimited capacity for self-renewal in response to mitogens, and are multipotent for the different postmitotic cell lineages of the CNS. 'NPCs' is used as a generic term that encompasses both CNS stem cells and progenitor or precursor cells.

Oligodendrocyte precursor cells (OPCs)—which are sometimes called NG2 cells[12]—are the most extensively studied NPCs. Adult OPCs are self-renewing and can generate astrocytes, neurons and Schwann cells as well as oligodendrocytes, so they could reasonably be regarded as a type of adult neural stem cell. Despite this claim, the relationship between OPCs and other adult neural stem cell populations remains uncertain at present, leading to ambiguities in terminology. In this Review, we use the term OPC to refer to a cell that typically expresses *NG2* and *OLIG* family genes, although we acknowledge that this distinction might be one of convenience rather than reflecting a clear biological division.

Immunomodulatory properties

The results of several studies in both rodent and non-human primate models of EAE indicate that NPCs, transplanted by either intrathecal or intravenous injection, promote bystander immunomodulation within the CNS via the release of soluble molecules, such as cytokines and chemokines. These cells are known to migrate into areas of inflammation within the CNS,[31,32,55,56] where their presence is associated with profound downregulation of effector functions of encephalitogenic inflammatory T cells, antigen-presenting dendritic cells, microglia, and macrophages.[23–29,31] Evidence exists that intravenously or subcutaneously transplanted NPCs might also exert immunomodulatory effects outside the CNS, at the level of peripheral lymphoid organs.[22,30] To date, no evidence indicates that OPCs transplanted either systemically or directly into the CNS are immunomodulatory.

Reparative mechanisms

OPCs transplanted directly into areas of CNS demyelination can elicit remyelination.[57–60] Remyelination has been successfully demonstrated by the use of both non-human and human cells, in focal lesions and in genetic disorders with widespread demyelination evident throughout the neuraxis.[60,61] Moreover, remyelination associated with OPC transplantation positively correlates with functional recovery, both behaviorally and electrophysiologically.[62–64] For a number of reasons, translation of these studies into clinical practice has not progressed

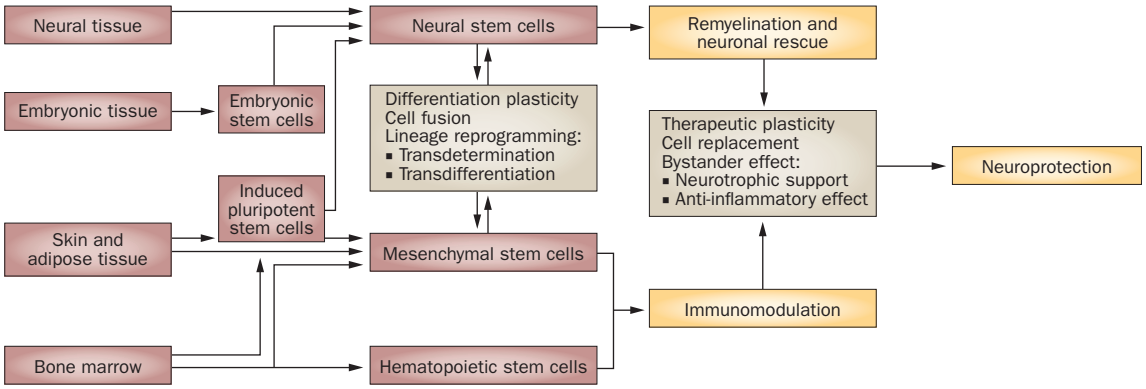

Figure 1 | Therapeutic plasticity of stem cells. Stem cell transplantation might promote neuroprotection by either cell replacement or bystander activity. This latter therapeutic behavior probably occurs as a result of the constitutive release of several molecules, such as cytokines, chemokines and integrins, by the stem cells. Stem cell bystander effects could also explain why somatic stem cells, which have low neural transdifferentiation capabilities, might efficiently promote CNS repair.

at the rate many would have predicted a decade ago. First, although OPCs migrate and proliferate within injured tissue, they are unable to survive and migrate through the normal intact adult CNS.[65] Therefore, individual lesions would need to be directly targeted for transplantation, which would restrict this approach to a handful of the most clinically relevant lesions. Second, obtaining large numbers of autologous human OPCs has proved to be less straightforward than was originally envisaged. Deriving OPCs from syngeneic human embryonic stem (ES) cells—and potentially in the future, autologous induced pluripotent stem cells (iPSCs)—remains a possible solution.[66,67] Last, it should be noted that much of the transplantation data are derived from toxin-induced models of demyelination or experimental animal models with genetically induced demyelination. Neither of these models will accurately reproduce the environments likely to be encountered by cells transplanted into MS lesions, although some experimental animal models—for example, shiverer mice and taiep rats—arguably provide conditions resembling chronic, minimally inflammatory MS plaques. These preclinical studies have, nevertheless, provided a wealth of information on the myelinogenic properties of various cell populations, and the factors that govern successful remyelination.

When injected directly into areas of demyelination, other types of NPCs have also been shown to efficiently remyelinate damaged axons.[68,69] This therapeutic effect depends, however, on the route of administration. NPCs delivered by the clinically more attractive intravascular or intrathecal routes demonstrate only modest differentiation and, consequently, evidence of remyelination after NPC transplantation is limited.[28,31,32] In this undifferentiated state, the cells exhibit not only potent anti-inflammatory properties (see above), but also a capacity to modulate growth factor release within the injured microenvironment.[32] Like MSCs, transplanted NPCs might potentiate the survival and regenerative properties of endogenous neural progenitor cells and could also provide a neuroprotective effect by increasing the bioavailability of major neurotrophins such as nerve growth factor, brain-derived neurotrophic factor, ciliary neurotrophic factor and glial cell line-derived neurotrophic factor. These neurotrophins might also contribute to the immunomodulatory properties of NPCs.[70] Taken together, these data suggest that NPCs transplanted intravenously and/or intrathecally could provide a therapeutic effect in MS by means of bystander 'neuroprotective' mechanisms rather than cell replacement.[34]

Cell sources

Somatic NPCs can be obtained from embryonic, fetal, neonatal or adult CNS tissues (Box 4). In serum-free cultures, supplemented with epidermal growth factor and fibroblast growth factor 2, NPCs proliferate almost indefinitely, growing either as multicellular free-floating spheres—neurospheres—or as single, layered, adherent cells. In both situations, the NPCs spontaneously differentiate into neurons, astrocytes or oligodendrocytes on withdrawal of mitogens from the culture medium. Of note, human adult NPCs possess decreased telomerase activity and shorter telomeres compared with rodent NPCs and have limited proliferation capacity during *in vitro* serial passaging. So far, only NPCs from fetal tissue-derived cells have been 'scaled up' under Good Manufacturing Practice (GMP) grade conditions, and are the only NPCs available for transplantation in clinical trials.[71] Furthermore, owing to human leukocyte antigen mismatch, transplantation of NPCs derived from fetal tissue into patients with MS requires immunosuppression. We are optimistic that autologous sources of NPCs and OPCs—potentially derived from iPSCs—will become available in the future.

Route of administration

Intrathecal injection is the preferred route of administration of NPCs. This recommendation reflects the fact that central immunomodulatory and, to a lesser extent, remyelinating effects are observed after intrathecal delivery of NPCs and that intrathecal injection is considered to be less invasive than direct injection into demyelinating lesions. Notably, however, the multifocality and pathological heterogeneity of MS lesions might limit the efficacy of such an approach.

Box 4 | Sources of neural stem cells for CNS repair

Embryonic stem cells
Embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of blastocyst-stage embryos. They possess two unique characteristics: indefinite self-renewal capacity, and pluripotency—the ability to generate all tissues of the body that are products of the epiblast lineage. *In vitro* propagation via continuous asymmetric cell division of ES cell-derived CNS-specific stem cells can be accomplished without accompanying differentiation. Protocols that avoid *in vivo* teratocarcinoma formation after ES cell transplantation are still lacking.

Induced pluripotent stem cells
Induced pluripotent stem cells (iPSCs) are a source of pluripotent stem cells that can be obtained by genetic reprogramming of somatic cells.[73,100,101] This genetic reprogramming is achieved by manipulating the somatic cells to express a set of four transcription factors: Oct-4, Sox-2, Krüppel-like factor 4 and c-Myc. Pluripotent stem cells could be derived from a patient's own cells. Autologous stem cells could be a future source of stem cells that do not provoke an immune response.

Adult neural stem/precursor cells
Adult neural stem/precursor cells (NPCs) are multipotent cells derived from embryonic, fetal, neonatal and adult CNS tissue. In serum-free cultures supplemented with epidermal growth factor and fibroblast growth factor 2, NPCs proliferate almost indefinitely. They spontaneously differentiate into neurons, astrocytes or oligodendrocytes after growth factor withdrawal.

Mesenchymal stem cells
Mesenchymal stem cells are a heterogeneous subset of stromal stem cells that can be isolated from many adult tissues. They can differentiate into cells of the mesodermal lineage and are currently defined as plastic adherent, multipotent fibroblast-like cells. These cells express CD73, CD90 and CD105 cell-surface markers, but do not express the hematopoietic markers CD14, CD34 and CD45, a trait shared by fibroblasts.[102]

In the near future, systemically injected NPCs, genetically modified or not, might represent a therapeutic option for patients with MS, as well as providing an important tool for delivering immunomodulatory, pro-remyelinating agents and/or neuroprotective drugs directly into the CNS.[72]

Patient selection

We recommend that proof-of-principle studies of NPC transplantation should be conducted in patients with early SPMS that is refractory to conventional therapy. Patients who already have marked disability but who possess sufficient residual function, which might be preserved by cell therapy, might be especially good candidates. This group has the advantage of being less heterogeneous, in terms of their disabilities, than relapsing patients. Therefore, quantifying disability progression in this group of patients is relatively straightforward. Trials with doses escalating up to 250×10^6 cells per patient are recommended for intrathecal delivery of NPCs in patients with SPMS. Until long-term safety of intrathecal NPC injection is proven, repeated cell injections should be avoided.

Other stem cell treatments

Embryonic and induced pluripotent stem cells

Many ethical issues are associated with the generation and use of ES cells; for example, harvesting of these cells results in the destruction of the embryos from which they are taken. The discovery of iPSCs might, in the future, circumvent these ethical considerations.[73] In the meantime, clinical trials using ES cells still face considerable safety challenges in view of the possibility of teratocarcinoma formation following transplantation. Owing to the occurrence of nonproliferative cysts in experimental animals receiving ES cells, the FDA has put on hold a Geron Corporation-sponsored clinical trial involving transplantation of human ES cell-derived OPCs into patients with acute spinal cord injury. Nevertheless, human ES cell-derived NPCs have shown similar immunomodulatory properties to rodent ES cell-derived NPCs in EAE models,[27] and can also be reliably manipulated to develop into cells of the oligodendrocyte lineage.[66,67] However, in our view, studies with ES cell derivatives in patients with MS are not yet justifiable, as limited data are currently available on their utility in MS treatments, and the tumorigenic potential of ES cell derivatives is still not completely understood. On the basis of our current knowledge, in the foreseeable future, human ES cell-derived oligodendocyte lineage cells will most probably be used in *in vitro* studies to examine the biology of human oligodendrocyte differentiation.

Olfactory ensheathing cells

Olfactory ensheathing cells (OECs) are differentiated glial cells with marked similarities to Schwann cells. In the peripheral olfactory system, these cells ensheath the axons of the first cranial nerve.[74,75] Although OECs normally wrap around very small axons without myelinating them, studies have shown that OECs can remyelinate larger axons with myelin that is indistinguishable morphologically and biochemically from myelin made by Schwann cells.[76,77] In particular, cell suspensions of acutely dissociated OECs from neonatal rats, when injected into areas of the spinal cord that have been demyelinated by ethidium bromide, remyelinate and enhance axonal conduction of demyelinated axons.[78] Moreover, canine, human or porcine OECs—isolated from the adult olfactory bulb—are capable of eliciting extensive functional remyelination following transplantation into the demyelinated CNS of rats.[77–81] OECs can be extracted from the olfactory mucosa of the human nasal cavity, which might, therefore, represent a readily accessible source of cells for transplant-mediated remyelination. OECs have been shown to integrate within areas of the brain in which astrocytic scars are present. These scars are a common feature of MS and, as a result, OECs have the potential to have widespread reparative effects in this disease.[82] Preliminary trials of autologous OEC transplantation into the injured spinal cord in both humans and dogs indicate that CNS transplantation of OECs is both feasible and safe.[83,84]

Schwann cell transplantation

The ability of Schwann cells to myelinate demyelinated axons in the CNS is well established.[85] Consequently, these cells have been used extensively as a means of driving exogenous remyelination. Moreover, a phase I clinical trial designed to evaluate Schwann cell transplantation

has been performed in patients with MS. Between July 2001 and April 2002, autologous Schwann cells were transplanted intracranially into single demyelinating lesions in three patients affected by SPMS, progressive relapsing MS or PPMS. This unpublished study demonstrated the safety of the transplantation procedure, but brain biopsies performed 5 months after transplantation provided no direct evidence that the transplanted Schwann cells had survived. For this reason, the study was discontinued in early 2003.[86]

More recently, interest has focused on the use of transplanted embryonic Schwann cell precursors and boundary cap cells to achieve remyelination. Both of these cell types represent a distinct PNS stem cell niche, since they seem to be less affected by CNS inhibitory factors than both neonatal and adult Schwann cells.[87] As a result, these cells are able to colonize and remyelinate more of the demyelinated CNS than either neonatal or adult Schwann cells.[88] These data, as well as the finding that neural crest-derived progenitor cells in peripheral tissues can give rise to Schwann cells,[89,90] open up new possibilities for Schwann cell-based therapy in demyelinating disorders. PNS stem cells can also be derived from ES cells,[91] but their therapeutic potential for remyelination remains to be demonstrated.

General issues

Exploratory clinical trials in MS

The unmet therapeutic needs in MS are clear evidence that continued exploratory trials of stem cell-based therapies are required. Although we agree with this point of view, we believe that these trials should be limited to patients with SPMS who have ongoing relapses and do not respond to conventional drug treatments, or patients with PPMS with ongoing CNS-confined inflammatory activity.[53] This suggestion is based on three principles agreed on by the STEMS consensus group participants. First, many effective therapies exist for patients with RRMS, and the use of concurrent immunomodulatory therapy would complicate any interpretation of the stem cell trial. Second, since early trials will primarily serve to determine the safety of stem cell transplantation in patients with MS, the risk–benefit ratio for patients who would otherwise have effective alternative treatment options is neither favorable nor ethically justifiable. Last, patients at the early stages of SPMS might still have some inflammatory lesion activity and, therefore, are more likely to have salvageable axons than are patients with advanced MS. Consequently, a biological rationale exists for studying stem cell transplantation in this patient population.

The environment that the stem cells are likely to encounter in patients with SPMS, such as multiple inflammatory foci, will probably be markedly different from the environment encountered in healthy volunteers. Therefore, we believe that only patients with SPMS should be enrolled in phase I MS stem cell trials. Phase I studies primarily focus on feasibility and safety issues, and investigators in these studies should examine surrogate markers such as imaging or electrophysiological outcome measures so as to gain a better understanding of the biological effects of stem cell therapies. Phase II studies are conducted in patients who have the disease or condition that the treatment in question is intended to treat. The objectives of conducting such studies include determination of the minimum effective dose of cells or drugs, or the dose that is sufficiently effective without undue toxicity. Patients with SPMS selected for early phase II studies should be free of hematological, hepatic, renal, cardiac or other serious diseases. Furthermore, only patients without concomitant diseases, and who are not taking other prescription drugs, should be included in late phase II studies, as they represent the patient population that would receive the investigational therapy if it gained approval. Surrogate markers for myelin repair and neuroprotection in MS are still lacking, and development of such biomarkers—biochemical, electrophysiological or imaging (non-conventional MRI and/or PET)—will help us to identify whether stem cell therapy is an effective treatment for patients with MS.

Recommended quality control tests

Quality control of GMP-grade cell production is mandatory for stem cell therapies aimed at treating patients with MS. The phenotype and karyotype of the stem cells, as well as their microbiological status, should be carefully assessed during *in vitro* cell manipulation. Information detailing the source of the stem cells should be freely available to the public and the scientific community, according to existing guidelines (European Medicines Agency and FDA).

Ethical and safety issues

Many important ethical and safety issues should be considered when devising stem cell therapies for MS.[92] For example, which patient groups should be included in or excluded from proof-of-principle trials? Also, do the potential benefits of the stem cell therapies outweigh the potential risks? Information regarding these safety and ethical issues should be incorporated in the informed consent documentation given to the prospective patient. In addition, owing to misperceptions that are widely held by patients that stem cell trials will cure them of their disease, we believe that stem cell trials should incorporate an assessment of the effectiveness of informed consent on patients' expectations of treatment. Patients with MS should be discouraged from approaching 'stem cell clinics' that do not follow the recommendations outlined above. International Society for Stem Cell Research guidelines should be strictly followed when undertaking stem cell-based treatments in human patients.[93]

Conclusions

A thorough understanding of any potential stem cell-mediated therapeutic mechanisms, other than tissue replacement, might result in the development of more-efficacious therapies for MS than are currently available. The potential of 'alternative' stem cell-mediated mechanisms such as immunomodulation or trophic

factor-mediated remyelination to confer neuroprotection in MS is of particular note. Systemic injection of genetically modified stem cells could, in the future, provide a means of delivering drugs to the CNS. Before this treatment option can be realized, however, we need to confront unsolved and challenging questions regarding the best way to regulate stem cell activity *in vivo*. Nevertheless, now is the right time to cautiously start investigating the safety of stem cell transplantation in neurological disorders such as MS, and to develop neuroradiological and other surrogate markers that will enable us to assess the outcome of stem cell therapies.

Review criteria

MEDLINE and the reference lists of papers were searched for articles published in the English language up to December 2009, using the following search terms: "multiple sclerosis", "experimental autoimmune demyelination", "demyelination", "stem cells", "neural stem/precursor cells", "mesenchymal stem cells", "hematopoietic stem cells", "remyelination", "olfactory ensheathing cells", "Schwann cells", "cell therapy" and "transplantation". Articles deemed relevant to the transplantation of stem cells in MS and its related animal models were selected for review.

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