Stem cells in Multiple Sclerosis

Dimitrios Karussis, MD, PhD, Ibrahim Kassis, PhD, Panayiota Petrou, MD

Department of Neurology and Laboratory of Neuroimmunology, and the Agnes-Ginges Center for Neurogenetics, Hadassah Medical Center, Hebrew University, Ein–Karem, Jerusalem, 91120, Israel.

Address for correspondence:
Prof. Dimitrios Karussis, MD, PhD
Head of the MS Center, Hadassah Medical Organization
Laboratory of Neuroimmunology,
Email: Dimitrios@hadassah.org.il
Tel: +972-2-6776939

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Abstract
To overcome the limited capacity of the CNS for regeneration, the theoretical alternative would be to use stem cells for more effective management of chronic degenerative and inflammatory neurological conditions, such as Multiple sclerosis. The precise reasons for the progression of disability in MS, despite the increasingly effective immunomodulating therapies, also remain largely obscure and puzzling. Recent findings indicate that progressive disease may be caused by axonal damage and atrophy, but can be also related to compartmentalized inflammation, that is purely amendable to the treatments and may be mediated by humoral mechanisms and cortical and deep gray matter damage. It seems therefore that in order to affect the progressive phases of the disease, alternative neuropotective modalities are needed and include stem cell cellular therapies.

Although the adult brain contains small numbers of stem cells in restricted areas, this intrinsic stem cell repertoire is small and does not measurably contribute to functional recovery. Embryonic cells carrying pluripotent and self-renewal properties represent the stem cell prototype, but there are additional somatic stem cells that may be harvested and expanded from various tissues during adult life. Stem cell transplantation is based on the assumption that such cells may have the potential to regenerate or support the survival of the existing, partially damaged cells. A great deal of positive data from numerous preclinical and few pilot clinical studies, support the possibility of induction of neurotrophic and neuroprotective effects by stem cell therapies, in MS. The mode of administration of the stem cells (and especially the intrathecal route that brings the cells in higher proximity to extensive areas of the CNS and has been long advocated and applied by our group) may be also crucial for the response of MS patients. Long-term safety data are still missing along with substantial evidence of clinical efficacy.
A. Background and rationale for stem cell therapies in MS

i. Multiple Sclerosis

Multiple sclerosis is a chronic inflammatory multifocal demyelinating disease of the central nervous system (CNS) that affects predominantly young adults. MS is the leading cause of chronic neurological disability in the young age. While its pathogenesis is still obscure, and multiple (genetic, environmental and infectious) factors seem to be involved in it, it is widely accepted that the final pathogenetic pathway is that of an autoimmune attack against myelin components (the outer layer/lining of all the axons in the CNS, which greatly facilitates the transmission of the electrical stimuli of the brain), which causes damage of myelin (demyelination) in multiple discrete areas of the CNS (the plaques of MS which are the pathological hallmark of the disease), dysfunction of multiple neuronal circuits in the CNS, leading therefore to neurological disability. Additional mechanisms have been more recently under covered, including a damage of the axons in the CNS and a degenerative process, which is probably the result of inflammation, and which causes accumulating and irreversible damage with time.

Extensive studies have provided strong evidence for neurodegeneration in MS, including the finding of amyloid precursor protein accumulation in neurons, a reduction in NAA/Cr ration in MR spectroscopy (MRS) which correlates well with the degree of disability, the finding of axonal ovoids/transected axons at the edge and the core of active lesions and of oxidative damage in mitochondrial DNA and impaired activity of mitochondrial enzyme complexes, the reduction in axonal density in normally appearing white matter (NAWM) early in MS and a more prominent reduction of axonal density in spinal cord NAWM in progressive MS patients. It is not clear which factors are responsible for the variability in the course of MS in different patients and the heterogeneity of the morphological alterations of
the CNS found by magnetic resonance imaging (MRI) or by histopathological evaluation, as well as a wide variability of the response to the immunomodulatory treatments. Possible explanations may include the complex genetic trait that translates into different immune abnormalities and/or increased vulnerability of CNS tissue to inflammatory insult or reduced ability to repair damage.

The precise reasons for the progression of disability in MS, despite the increasingly effective immunomodulating therapies, also remain largely obscure and puzzling. Recent findings indicate that progressive disease may be caused by the previously mentioned-axonal damage and atrophy, but can be also related to compartmentalized inflammation, that is purely amendable to the treatments and may be mediated by humoral mechanisms and B-cells and cortical (sub-meningeal) and deep gray matter damage.


All the currently approved treatments for MS are targeting the immune system aiming to suppress the inflammatory components if the disease in a non-specific manner (generalized immunosuppression) and/or in a more restricted way (immunomodulation). In both cases, these treatments are only preventing at some extent the appearance of new relapses and the progression of the disease, but cannot reverse existing disability.
It seems therefore that in order to affect the progressive phases of the disease, different immunomodulating modalities (ie more effective in suppression of the localized deep inflammation and in downregulation of humoral mechanisms) and/or neuroprotective approaches are essential. These may include cellular therapies, including stem cell treatments that may be more effective in suppression of compartmentalized inflammation and in induction of neurotrophic effects, neuroprotection and possibly even enhance regeneration.

**ii. Stem cell therapy for Multiple Sclerosis**

Stem cells are a diverse group of multipotent cells. In general, these cells are relatively undifferentiated and unspecialized, and can give rise to the differentiated and specialized cells of the body. All stem cells exert two characteristic features: i. the capacity for self renewal which preserves a pool of undifferentiated stem cells, and ii. the potential for transdifferentiation (the ability to produce various differentiated cell types). There are different kinds of stem cells that can be isolated from embryonic and adult tissues. Embryonic stem cells (ESCs) which are the prototype of all stem cells, are cells derived from the inner cell mass of embryos at the blastocyst stage (5–9 days after fertilization). The only source for human stem cells is from embryos obtained from in vitro fertilization.

A significant breakthrough in the stem cells research was the discovery of stem cells which reside in various body tissues (including the brain), during the adult life. These are defined as adult stem cells and represent a more differentiated cell population than ESCs. They can be isolated from various tissues, including muscle, adipose tissue, CNS (neural stem cells [NSCs]) and bone marrow (mesenchymal stromal cells [MSCs] and hematopoietic stem cells [HSCs]). All of the previously described stem cells carry a potential for specific and non-specific tissue repair.
B. Different types of stem cells

i. Embryonic stem cells

ii. Adult neural stem cells (aNSC)

Adult NSC or “CNS neural stem cells” are cells that are cultured as sparse adherent cells or as aggregates of floating cells called “neurospheres” in serum-free medium on a non-adherent surface in the presence of epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF-2). NSCs express markers as: Nestin PSA-NCAM, and Sox2. These cells as their name implies can give rise to neural, astrocytic and oligodendrocytic precursors that can in turn may differentiate into neurons, astrocytes and oligodendrocytes. Based on their ability for neural and glial cell generation, NSCs were tested in the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). In a study by Pluchino et al. it was shown that adult neural stem cells cultured and injected into EAE-mice—intravenously (iv) or intracerebroventricularly (icv), could migrate into the demyelinating CNS area and differentiate into mature brain cells. It was noticed in this study, that intrinsic oligodendrocyte progenitors were significantly increased, in the CNS of the NSC-injected animals. Clinically, EAE symptoms were strongly down-regulated in the transplanted animals. In additional studies by Einstein et al, and Ben-Hur et al. from our Medical Center and laboratory at Hadassah Neurology department, it was found that transplanted neural precursor neuroshperes (icv) migrated into the inflamed white matter in EAE, attenuated the severity of clinical signs and reduced brain inflammation. In these studies, it was shown that when neural precursors were administered intravenously, EAE was suppressed by a peripheral immunosuppressive effect, which inhibited T-cell activation and proliferation in the lymph nodes. Moreover, the transplanted neural precursor cells could down-
regulate the inflammatory brain process in situ, as indicated by the reduction in the number of perivascular infiltrates and of brain CD3+ T cells, a reduction in the expression of ICAM-1 and LFA-1 in the brain and an increase in the number and proportion of regulatory T cells in the CNS. The later may indicate an active immunoregulation induced by the NPC.

Despite these promising results, that place the NSC as the optimal adult stem cell population for cell replacement therapy in CNS diseases, these cells are associated with significant drawbacks. First, the difficulty to culture neurospheres from regions of the adult brain that do not normally undergo self-renewal. Second, although NSC can be propagated for extended periods of time and differentiated into both neuronal and glial cells, studies suggest that this behavior is induced by the culture conditions of the progenitor cells, and seems to be restricted to a limited number of replication cycles in vivo. Furthermore, neurosphere-derived cells do not necessarily behave as stem cells when transplanted back into the brain. Additional concerns include the potential for immune rejection of NSC, the danger of tumor development in the host brain and various ethical aspects related to the donor tissue origin.

**iii. Induced Pluripotent Stem (iPS) cells**

IPS are adult cells that have been genetically reprogrammed to an embryonic stem cell–like state by making these cells express genes and factors important for maintaining the defining properties of embryonic stem cells. In 2006 Yamanaka and colleagues showed that induced pluripotent stem (iPS) cells can be generated from mouse fibroblasts by the retrovirus-mediated transfection of four transcription factors, namely Oct3/4, Sox2, c-Myc, and Klf4. These iPS cells were found to have the embryonic stem cells morphology and several biological features including gene
expression, and teratoma formation. In 2007, the same group presented similar result
for human iPS cells\textsuperscript{97}. They demonstrated the generation of iPS cells from adult
human dermal fibroblasts with the same four factors: Oct3/4, Sox2, Klf4, and c-Myc.
Human iPS cells were similar to human embryonic stem (ES) cells in morphology,
proliferation, cell surface antigens, gene expression profile and telomerase activity.
These cells were shown capable of differentiating into cell types of the three germ
layers in vitro and in teratomas.

Recently, the generation of patient-specific cells from induced pluripotent stem cells
(iPSCs) has emerged as a promising strategy for the development of autologous cell
therapies\textsuperscript{98}. In a hypomyelinated mouse model it was shown that oligodendrocyte
progenitor cells (OPCs) derived from IPSc lines induced a high degree of
remyelination and in this myelin-defective mouse\textsuperscript{99}. However, the differentiation
protocols are still inefficient and not easily reproducible and require over 120 days in
culture. In 2014, Douvaras et al reported a highly reproducible protocol to produce
oligodendrocyte progenitor cells (OPCs) and mature oligodendrocytes from iPSCs\textsuperscript{100}.
The major elements of the protocol include adherent cultures, dual SMAD inhibition,
and addition of retinoids from the beginning of differentiation, which lead to increased
yields of OLIG2 progenitors and high numbers of OPCs within 75 days\textsuperscript{100}. Morover,
the authors reported the generation of integration-free iPSCs from primary
progressive MS (PPMS) patients and their efficient differentiation to
oligodendrocytes. PPMS OPCs were found to be functional, as demonstrated by in
vivo myelination in the shiverer mouse mouse model\textsuperscript{100}. These results represent
encouraging advances towards the future development of autologous cell therapies
using iPSCs.
Hematopoietic stem cells (HSC) are the main stem cells population of the bone marrow. These cells typically express the surface markers phenotype of CD34+, CD133+, CD45+, CD38−. HSC are the precursor cells that give rise to all types of blood cells, including T-cells, B-cells, NK-cells, macrophages, red blood cells, granulocytes and other monocytes. Several studies have shown the ability of HSC to transdifferentiate into CNS cells including neurons, astrocytes and oligodendrocytes. Hematopoietic stem cells transplantation (HSCT) or bone marrow transplantation (BMT) is widely used in hematological malignancies. During the last few years HSCT or BMT has been also applied for the treatment of autoimmune diseases as multiple sclerosis. The rationale for this application has been provided by studies from our group which have shown that high dose cyclophosphamide for the elimination of immunocompetent lymphocytes, followed by syngeneic BMT rescue, could suppress chronic EAE and induce tolerance to the immunizing antigens. Several small open trials over the last two decades have shown the efficacy of autologous HSCT in suppressing the inflammatory activity in patients with severe MS. Younger patients with relapsing forms of MS are most likely to respond to the treatment. The most recent of the open trials in 26 patients with active relapsing MS and a long median follow-up period (186 weeks) showed an overall event-free survival of 78.4% at 3 years. Progression-free survival and clinical relapse-free survival were 90.9% and 86.3%, respectively. Adverse events were consistent with expected toxic effects associated with HDIT/HCT, and no acute
treatment-related neurologic adverse events were observed. Improvements were
noted in neurologic disability, quality-of-life, and functional scores.

The apparent problem of the use of HSCT or BMT in autoimmunity is the need for
strong (lethal) immunosuppression, which is associated with significant morbidity and
mortality. The use of lower doses of immunosuppression is not only less efficient but
may actually provoke relapses of EAE. In addition, despite the significant effects
of such protocols in suppressing the inflammatory activity, still the clinical effects
were not equally impressive, especially in the progressed stages of MS.

In recent reports from two groups, it was shown that despite the efficacy of HSCT
in MS patients in terms of elimination of the inflammatory lesions in the MRI and
clinical stabilization (progress free patients >60% in 3 years), still this treatment did
no prevent continuation of brain atrophy and did not induce any functional
improvement in most of the patients, especially in those with high disability scores.

Moreover, the procedure-related mortality (solely attributed to the cytotoxic
conditioning protocol) in these studies exceeded may be in some case as high as 5% which
seems to be an unacceptable risk for the majority of the MS patients. A
randomized controlled clinical trial with HSCT versus immunosuppressive modalities
in MS, is underway. A recent report of a small group (21 patients, nine were
randomized in the AHSCT and 12 in the MTX arm) from this randomized study
(Autologous hematopoietic stem cell transplantation in multiple sclerosis: A phase II
trial. Neurology. 2015 Mar 10;84(10):981-8) showed that AHSCT reduced by 79% the
number of new T2 lesions as compared to mitoxantrone 20mg/month (rate ratio
0.21, p = 0.00016). It also reduced Gd+ lesions as well as the annualized relapse
rate. No difference was found in the progression of disability. The authors concluded
that AHSCT is significantly superior to cytotoxic treatment (mitoxantrone) alone, in
reducing MRI activity in severe cases of MS.
v. Mesenchymal stem cells (MSC)

MSCs are another important member of the bone marrow stem cell repertoire. These cells are described as nonhematopoietic stromal cells and their classical role is to support the process of hematopoiesis and HSC engraftement and to give rise to cells of mesodermal origin (Figure 2.4), such as osteoblasts, adipocytes and chondrocytes. The ability to differentiate to another lineages like the neuronal one is controversial and will be discussed later. These cells do not have a specific surface marker profile, but it is widely accepted that they are negative for CD34, CD45 and CD14, and positive for CD29, CD73, CD90, CD105 and CD166.

a. In vitro neuroprotective features of MSCs

Several studies suggested that the neuroprotective potential of MSC is mediated by the production of neurotrophic factors that support neuronal cell survival, induce endogenous cell proliferation and promote nerve fiber survival and even regeneration at sites of injury. For in vitro studies, several neural-like cell models (e.g. PC12 cells, DRG cells, SH-SY5Y neuroblastoma cells) were utilized to depict the neuroprotective features of MSC. In a study by Scuteri et al, MSC obtained from adult Sprague-Dawley rats were co-cultured with DRG post-mitotic sensory neurons obtained from rat embryos at day E15. Co-cultures were maintained for 2 months. The co-culture with MSC allowed long time survival and maturation of the DRG cells. The degree of survival and maturation of the DRG neurons was significantly lower when fibroblasts were used in the co-culture instead of MSC. In a recent study by the same group it was found that MSCs are able to prolong the survival DRG neurons mainly by inhibiting proteolytic enzymes, and in particular the pathway of metalloproteinases, a group of proteins that are involved in many neuronal processes, including their survival. Lu et al, used MSC isolated from adipose tissue to evaluate their neuroprotective potential on PC12 cells challenged with glutamate to cause excitotoxicity-induced apoptosis. In this setting, MSC secreted neurotrophic factors...
including vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). Addition of MSC-conditioned medium on the culture, had a protective effect on excitotoxicity-injured PC12 cells, as indicated by the increased cell viability, decreased number of TUNEL-staining positive nuclei and lowered caspase-3 activity. Additionally, coculture of MSC with SH-SY5Y neuroblastoma cells enhanced the survival and neurite outgrowth of these cell lines. In a study by Crigler et al, screening of cDNA library of human MSCs was performed and revealed a high expression of transcripts encoding NGF and BDNF.

b. In vivo neuroprotective features of MSC

The indications that MSCs may trans-differentiate into neural-like cells and their ability to induce neurogenesis and neuroprotection, support the possibility that MSC-based therapy may be efficacious in the management of neurological diseases. In general, MSCs seem to share with other types of stem cells the property of inducing and promoting a neuroprotective and neurotrophic environment, which was described by an elegant and pioneer study by Langer and colleagues. In this study, the researchers utilized a scaffold seeded with neural stem cells to promote functional recovery after spinal cord injury. The positive effect of the scaffold loaded with NSC on the neuronal repair and recovery was attributed to trophic effects of the NSC rather than cellular replacement at the site of injury. In the case of MSCs, similarly to the effects of other types of adult stem cells, the putative mechanisms of neuroprotection/neurorepair may include the production of neurotrophic factors that support neuronal cell survival, the induction of endogenous neuronal stem cells proliferation and the promotion of neuroregeneration at the site of injury. These neuroprotective effects were evaluated in different animal models. In a model of injured neurons of the optic tract in rats, it was found that MSC exert neuroprotection leading to improvement of the survival of a significant proportion of
the axotomised retinal ganglion cells. The MSC used in this study were found to secrete immunomodulatory and neurotrophic factors including TGF-beta, CNTF, BDNF and NT-4. In a model of induced focal demyelination of the spinal cord, intravenous and intracerebral infusion of MSC resulted in remyelination. In a model of stroke, the intravenous administration of MSCs resulted in improving functional recovery while reducing the apoptosis of cells in the injured tissue. Moreover, an increase in the expression of basic fibroblast growth factor (bFGF) and endogenous neurogenesis was observed. In other studies, hippocampal administration of MSCs in immunodeficient mice stimulated the proliferation, migration and differentiation of the endogenous neural stem cells, which survived as differentiated neural cells via their secretion of various trophic factors, including nerve growth factor (NGF), vascular endothelial growth factor (VEGF), ciliary neurotrophic factor (CNTF) and basic fibroblast growth factor (FGF-2).

Although all of these and other studies presented a potent and clear neuroprotective effects in different neurological disease models, we should take in considerations the limits and questions still opened regarding the effect of these cells in vivo and which may be very relevant for later stage with human use. There are still open questions regarding the survival and viability of the engrafted cells with the different injured tissues; the doses that be used to get the maximum effect and how do these cells act within the niche it delivered into. In a study by Lepski and colleagues they evaluated the survival and neuronal differentiation of MSC administrated into the rodent brain. They found that survival and differentiation of MSCs is strongly dependent upon a permissive microenvironment. Identification of the pro-neurogenic factors present in the hippocampus could subsequently allow for the integration of stem cells into “restricted” areas of the central nervous system. Moreover, several studies indicate that ageing can affect the proliferation and differentiation capacities of MSCs. It has been shown that long term culture may result in senescence, loss of differentiation capacity and ultimate growth arrest. We should pay attention that different results
concerning in vivo behavior of MSC can be attributed to species, gender and donor age of animals used in these studies and as well to differences in the conditions of cell culture.

c. In vitro immunomodulatory features of MSCs

As reported by previous and our studies, MSCs have important immunomodulating properties; they were found to suppress in vitro T- and B-cell functions and NK cells 64. MSCs suppress the proliferation of both CD4+ and CD8+ T lymphocytes, as well as of NK cells, whereas they didn not show an equal effect on the proliferation of B-lymphocytes 74. Although the exact mechanisms of the immunosuppressive effects of MSCs are not yet fully clarified, two main mechanisms have been suggested: i. humoral mechanisms, involving the production of soluble factors and, ii. cell-to-cell contact dependent mechanisms 75, 76. Several soluble factors have been suggested to be involved, including TGF-β1 77, IFN-γ 74, indoleamine 2,3-dioxygenase (IDO) 78 and prostaglandin E2 79.

d. In vivo immunomodulatory properties of MSC

The immunomodulatory and neuroprotective properties of MSCs in vitro were confirmed by us and other groups mainly in the model of experimental autoimmune encephalomyelitis (EAE), an induced autoimmune inflammatory demyelinating paralytic disease that serves as an animal model of multiple sclerosis. The in vivo immunomodulatory effects of MSCs were also documented in additional animal models such as in GVHD models and other induced autoimmune diseases 80, 81. Based on their in vitro properties one could assume that MSCs may downregulate in vivo the autoimmune attack to myelin antigens in this model and possibly promote nervous tissue repair or neuroprotection. Zappia and colleagues demonstrated that the injection of syngeneic MSC, indeed ameliorated the clinical severity of the disease in a mouse model of acute monophasic EAE (induced in C57bl mice using
the myelin oligodendrocyte protein – MOG\textsubscript{35-55} and reduced demyelination and leukocytes infiltration of the CNS\textsuperscript{82}. The findings were explained by the induction of T-cell anergy by MSC-treatment. In the study by Zhang et al., it was shown that intravenous administration of MSCs could suppress the disease in a relapsing-remitting model of EAE induced in SJL mice\textsuperscript{83}. MSCs migrated into the CNS where they promoted BDNF production and induced proliferation of a limited number of oligodendrocyte progenitors. Evidence of neuroprotection in EAE following MSC treatment was also shown by Chops et al\textsuperscript{84}, accompanied by indications of in vivo neural differentiation of the transplanted cells\textsuperscript{84}. Gerdoni et al, used in his study the relapsing-remitting model of EAE that was induced with the preteolipid protein (PLP) in SJL mice\textsuperscript{85}. Intravenously treated mice with MSC had a milder disease and developed fewer relapses than the untreated control animals\textsuperscript{85}. These results were coherent with histopathological findings that included decreased inflammatory infiltrates and reduced demyelination and axonal loss in the brains of the treated mice. No evidence for in situ transdifferentiation of the transplanted cells was documented\textsuperscript{85}. In studies from our group, a model of chronic EAE (more reminiscent of human MS) was used and the effect of MSC transplantation via additional routes (both intravenous and intraventricularly, directly into the brain and CSF), was evaluated. Although in previous studies, the suggested mechanism of suppression of EAE following intravenous injection of MSCs was suggested to be that of induction of peripheral immunomodulation/anergy\textsuperscript{82,85}, in our experimental setting, we verified the advantages of direct injection of MSCs into the ventricles of the brain, where they induced a more prominent reduction in infiltrating lesions, indicating an additional in situ immunomodulation. The peripheral immunomodulatory effects of MSCs are probably equally important and the migratory ability of these cells to the lymph nodes and other lymphatic organs (when injected intravenously), shown in this work, argue in favor of such –additional- peripheral mechanism. GFP labeled MSCs injected via the iv route, migrated into the lymph nodes, spleen, lungs and brain. These findings
are in agreement with previous studies regarding the bio-distribution of iv-injected MSC \(^{86, 87}\). Long-term engraftment of the cells was evidenced and the injected cells were viable after 30-40 days of transplantation.

It is therefore logical to assume that the main immunomodulatory activity of MSCs is exerted in the peripheral lymphoid organs where MSCs migrate following i.v. administration, inhibiting T-cells homing in the CNS \(^{59, 82}\). In addition to these peripheral effects, MSCs migrating to the CNS following i.v. and i.c.v injection may also locally further modulate the CNS autoimmune process, stimulate endogenous neurogenesis and protect neurons and oligodendrocytes, by similar paracrinic and neurotrophic mechanisms \(^{59}\).

The above-discussed in vivo experiments in EAE utilized the model of autologous MSC transplantation. This setting is logically considered more convenient for clinical transplantation since it does not hold any risks of rejection of the transplanted cells. However, in clinical reality, it is not always feasible that the patient can serve as a donor, due to his/her progressed medical condition. Moreover, if genetic factors are involved in the pathogenesis of MS, it would be preferable to avoid transplantation of stem cells carrying a -putatively- defective genome. Therefore, the possibility of allogenic MSC transplantation (using MSCs obtained from healthy donors) might be considered, especially since MSC were shown to “escape” rejection by “masking” parts of the immune response, such as the complement system \(^{88}\). Three main mechanisms contribute to this “immune-privileged” status of MSCs: I) MSCs are hypoimmunogenic, often lacking MHC-II and costimulatory molecules expression \(^{75}\); II) MSCs prevent T-cell responses indirectly through modulation of the dendritic cells and directly through downregulation of the NK, CD8+ and CD4+ T-cell functions \(^{64}\); III) MSCs induce a suppressive local microenvironment through the production of prostaglandins and interleukin-10 as well as by the expression of indoleamine 2,3,-dioxygenase, which depletes the local milieu of tryptophan \(^{64}\). A possible attractive explanation of the reported efficacy of allogeneic MSC-transplantation may involve a
mechanism of a “single hit” (probably immunomodulatory or neurotrophic, in its nature), directly following the injection of MSCs, and before any putative rejection process may take place. In support to such possibility come recent studies, which consistently reported that MSCs induce significant beneficial clinical effects and potent immunomodulation and neuroprotection mediated by the production of neurotrophic factors and/or through the recruitment of local/intrinsic CNS precursor cells \textsuperscript{89-92} or paracrinic mechanisms. Rafei and colleagues showed that the suppressive effect of MSCs on the encephalitogenicity of Th17 CD4-T cells was achieved through a metalloproteinase-mediated paracrine proteolysis of CCL2 leading to an increase in the programmed cell death, mediated by ligand-1 (PDL1) \textsuperscript{90}. Indeed, others reported that interactions between PDL1 on MSCs and PD1 on T-cells are involved in the inhibition of T-cell \textsuperscript{93} and B-cell proliferation \textsuperscript{94}, suggesting an interaction between MSC and lymphocytes which requires both cell contact and paracrine effects. Some of the immunomodulatory effects of MSCs are species specific as indicated by the finding that IDO is involved in the immunosuppressive activity of human MSCs and iNOS in that of mouse MSCs \textsuperscript{95}.

e. Clinical experience with MSC in MS

Due to the above mentioned practical advantages of MSC, bone marrow MSC are, to date, the most commonly used stem cell population in clinical trials, with the exception of hematopoietic stem cells, especially regarding the treatment of neurological diseases in general and MS specifically. As these cells seem to be able to cross the BBB (blood–brain barrier), the need for invasive intracerebral surgery can be avoidable neurological diseases and—at least—the peripheral systemic administration has been proven a rather safe and efficient way for cell delivery in humans \textsuperscript{101}. In a recent meta-analysis of clinical trials utilized intravascular delivery of MSC (intravenously or intra-arterially) testing immediate events (toxicity, fever), organ system complications, infection, and long term adverse events (death,
malignancy) it was found that peripheral MSC administration is safe. The data revealed from randomized control trials did not detect any association between acute infusional toxicity, organ system complications, infections or deaths. However, the extent to which MSC can be directed to a neural or other than mesodermal cellular fate either ex vivo or in vivo following transplantation still a point of controversy.

For clinical trials, isolated MSC should be produced according to Good Manufacturing Practice (GMP). The culture process should be reproducible and efficient. According to guidelines of the International Society for Cell Therapy (ISCT) the minimal criteria to define human MSC are: 1) MSC must be plastic-adherent when maintained in standard culture conditions using tissue culture flasks; 2) more than 95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells should be negative for the CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA class II markers. 3) the cells must be able to differentiate to osteoblasts, adipocytes and chondroblasts under in vitro differentiating conditions. Safety is the major concern during the culture process as well as quality control of these cells. Several levels of quality control during the production of MSC are necessary. These should include various microbiological tests (including bacterial, viral and mycoplasma detection and LPS levels) and genetic-karyotype testing to exclude contaminations and genetic transformations or instability. The main source for MSC used for clinical trials is the bone marrow compartment, but MSCs can be also harvested by other tissues such as the adipose tissue and the umbilical cord. The culture process is also an issue of major consideration, since the culture can be started from either unfractioned (whole BM) or fractioned cells (mononuclear fraction of BM after gradient density). The medium of choice for culturing is of equally high importance for the efficacy and safety of MSCs. Generally, Dulbecco’s Modified Eagle’s medium (DMEM) or alpha-minimal essential medium are used for culturing with the addition of foetal bovine
serum (FBS), foetal calf serum (FCS), human serum, plasma and platelets lysates, with the addition of growth factors as FGF. The use of serum is one of the controversial parameters of the culture having an impact on the batch-to-batch variability and the risks of contamination. The use of chemical-defined, xeno-free, serum-free medium (SFM) may provide a preferable solution. The final product of MSC that will be used for transplantation should be tested microbiologically to detect the presence of aerobic and anaerobic microbes, mycoplama and endotoxin levels and genetically (karyotypic profile and stability) before the administration to patients. Viability of the cells should be also checked and be more than 80%.

The lack of standardization for MSC isolation and culturing has delayed the progress in the field of MSC use in human diseases since the comparison of results from different laboratories was sometimes impossible. Any differences in the culture conditions might selectively favor the expansion of different subpopulation. Based on morphology, several distinct cell types can be distinguished: spindle-shaped fibroblast like cells, large flat cells and small round-cell subpopulations. The quality of preparations from different protocols are various and the cell products therefore heterogeneous. The source and quality of the starting material, culture media used, the use of animal serum, cytokines supplements, initial seeding cell density, number of passages upon culture and even type of cell culture dishes, all have a significant influence on the cell populations that are finally produced. Therefore, there is an urgent need for the development of standardized cell culture reagents and products, common guidelines and standards (SOPs) for MSC preparations and of molecular and cellular markers to define subpopulations with different potentials. Only by these standardizations, the potential of MSC in the treatment of different human diseases will be effectively evaluated.
f. Modified MSC

MSCs can be manipulated to secrete higher quantities of neuronal growth factors and therefore pushed towards a neuronal transdifferentiation ("neuralization") and gain higher neurotrophic and neuroprotective effects. Such method has been developed by Brainstrom Co, and used by our group in a recently completed trial in ALS with very promising results (Petrou, Karussis et al, manuscript submitted). The MSCs are transformed to neurotrophic factor-secreting cells (MSC-NTF), following a medium-based differentiation process. These cells not only secrete NTFs such as GDNF and brain derived neurotrophic factor (BDNF), but also express astrocytic markers. MSC-NTFs can migrate better towards brain lesions and were shown effective in several models of neuronal damage, such as the 6-hydroxy dopamine model for Parkinson’s disease\textsuperscript{105}, models for Huntington's disease\textsuperscript{106, 107}, models of optic nerve damage\textsuperscript{108}, and in the model of multiple sclerosis, EAE\textsuperscript{109} and after sciatic nerve injury\textsuperscript{110}. A trial in MS with these modified MSCs is under preparation in our Center.

Another group, has used a different method to produce bone marrow mesenchymal stem cell-derived neural progenitors (MSC-NPs) as an autologous source of stem cells. MSC-NPs have stronger neural progenitor and immunoregulatory properties, and a reduced capacity for mesodermal differentiation and showed efficacy in the animal model of EAE\textsuperscript{111}.

g. Pilot Clinical trials with stem cells in MS

Phase I/II safety studies with MSC or bone marrow-derived cells have been performed in multiple sclerosis\textsuperscript{112-114}. Overall, MSCs given intravenously or intrathecally were well tolerated, with some preliminary evidence of efficacy\textsuperscript{112}.

On the basis of the preclinical data from our studies and the cumulative data from other centers, an exploratory clinical trial with autologous bone marrow–derived MSCs in 15 patients with intractable MS, was initiated at Hadassah\textsuperscript{112, 115}. In this
trial, based on the data in EAE models (indicating probably two distinct mechanisms of action by the two different routes of MSC-administration), a combined intrathecal and intravenous administration was used to maximize the potential therapeutic benefit by accessing the CNS through the cerebrospinal fluid and the systemic circulation. In 9 patients, MSCs were labeled with the superparamagnetic iron oxide magnetic resonance imaging (MRI) contrast agent ferumoxides (Feridex™) to track cell migration after local grafting.

Follow up of the patients for 6 months, showed that the mean EDSS (disability) score of the transplanted MS patients, improved from 6.7±1.0 to 5.9±1.6. MRI visualized the MSCs in the occipital horns of the ventricles, indicative of the possible migration of the labeled cells in the meninges, subarachnoid space, and spinal cord. Immunological analysis at 24 hours post-transplantation revealed an increase in the proportion of CD4+CD25+ regulatory T cells, a decrease in the proliferative responses of lymphocytes, and the expression of CD40+, CD83+, CD86+, and HLA-DR on myeloid dendritic cells.

Since this was a pilot, feasibility study, the most important finding was the acceptable safety profile of transplantation of autologous stem cells from the bone marrow in patients with MS. None of the patients experienced significant adverse effects during the 6- to 25-month observation. The follow-up MRI 1 year after transplantation did not reveal any unexpected pathology or significant new activity of the disease. Several clinical trials in non-neurological diseases have also indicated that intravenous administration of MSCs is a safe procedure. The study in Hadassah Center additionally showed an acceptable short-term safety profile of the intrathecal route of administration of stem cells at doses of up to 70 million cells per injection per patient. The intrathecal approach (which was supported by the pre-clinical data from our group showing that this route of administration could induce superior neurotrophic and neuroprotective effects) may be more advantageous for cell-based therapies in neurological diseases, in which the areas of tissue damage are widespread
throughout the neuroaxis, since it may increase the possibility of migration of the injected cells to the proximity of the CNS lesions. The injected cells may circulate with the flow of the cerebrospinal fluid and have a better chance of reaching the affected CNS areas. However, the optimal route of stem cells administration in general and particularly MSC administration in patients with neurological diseases remains debatable and is currently tested in a large double blind trial that is running in our Center at Hadassah.

In the above-described trial, MSC were labeled with iron particles for MRI analysis. Such labeling of MSCs with the commercially used paramagnetic material, Feridex, was shown to be safe and had no negative effect on the functional (immunomodulatory and neurotrophic) properties of MSCs. It seems therefore that Feridex may be used for the tracking of this type of stem cells in clinical applications, without compromising their major functional properties.

Additional clinical trials explored the safety, and therapeutic benefit of intrathecal injection of ex-vivo expanded autologous bone marrow-derived mesenchymal stem cells in patients with advanced multiple sclerosis. In the later study, assessment of the patients at 3-6 months revealed an improvement in EDSS score in 5/7, stabilization in 1/7, and worsening only in 1/7 patients. Vision and low contrast sensitivity testing at 3 months showed improvement in 5/6 and worsening in 1/6 patients. These preliminary results indicate additional (to the Hadassah trial) hints of clinical -but not radiological- efficacy and evidence of safety with no serious adverse events. A more recent phase 2a study in 10 patients with secondary progressive MS showed an improvement in visual acuity and visual evoked response latency, accompanied by an increase in optic nerve area, following intravenous transplantation of autologous MSC. Although, no significant effects on other visual parameters, retinal nerve fibre layer thickness, or optic nerve magnetization transfer ratio, were observed, This study provides a strong indication for induction of tissue repair with MSC transplantation, in humans.
Another small pilot trial recruited 25 patients with progressive MS with an EDSS score of up to 6.5, unresponsive to conventional treatments\textsuperscript{119}. The patients received a single intrathecal injection of ex-vivo expanded MSCs (mean dose: 29.5×10^6 cells). Short-term adverse events of the injection included transient low-grade fever, nausea/vomiting, weakness in the lower limbs and headache. No major delayed adverse effect was reported. The clinical course of the disease improved in 4 patients, deteriorated in 6 and did not change in 12 patients.

**h. Ongoing or recently completed clinical trials with mesenchymal stem cells in MS**

A small, open-label, phase I clinical trial led by Dr. Jeffrey A. Cohen at Cleveland Clinic tested the ability of an individual’s own mesenchymal stem cells to downregulate inflammatory mechanisms and to augment intrinsic tissue repair processes in people with relapsing forms of MS. They were given intravenously (infused into the vein). This trial, which was designed to evaluate safety and not designed to determine benefits, was completed and preliminary results were presented in the ECTRIMS meeting (September 2014), suggesting that this approach was safe and warrants a phase 2 trial, which is now in planning stages.

A small, open label, phase I stem cell trial has begun at the Tisch MS Research Center of New York using individuals’own mesenchymal stem cells to derive more specific stem cells called “neural progenitor cells.” The cells are expanded in the laboratory and then injected into the space around the spinal cord (intrathecal). The goal is to inhibit immune mechanisms and to augment tissue repair.

Another small, open label, phase I trial of stem cells derived from placenta (known as “PDA-001” manufactured by Celgene Cellular Therapeutics) was completed in 2014, and results suggested this approach was safe. The study involved 16 people with relapsing-remitting or secondary-progressive MS at sites in the U.S. and Canada. This study was designed to evaluate safety and not designed to show
effectiveness. In the published paper, the researchers comment that the next step, a proof-of-concept clinical trial, is planned.

A placebo-controlled, phase II stem cell trial involving people with secondary-progressive MS and primary progressive MS has begun at Frenchay Hospital in Bristol, United Kingdom, testing the benefits and safety of using individuals’ own bone marrow cells. The cells are extracted and then given by intravenous infusion immediately or one year after the extraction. The goal is to inhibit immune mechanisms and to augment tissue repair.

The largest and only randomized double blind-control trial in 48 MS patients is currently running at Hadassah Medical Center (PI: Dimitrios Karussis) and is aimed to answer the critical questions of efficacy, neuroregeneration potential and optimal route of administration of the mesenchymal stem cells and the added value of repeated injections.

C. CONCLUSIONS

In conclusion, a decade of intensive preclinical and clinical research has greatly advanced our understanding of the role of stem cells in neurological diseases in general and specifically in MS, but has not yet fully clarified the picture. A great deal of positive data support the possibility of neuronal regeneration and neuroprotection. The mode of administration of the stem cells (and especially the intrathecal route that brings the cells in higher proximity to extensive areas of the CNS and has been long advocated and applied by our group) may be also crucial for the response of MS patients.\textsuperscript{14, 25} Long-term safety data are still missing along with substantially proven efficacy. Stem cells do not yet represent a panacea for all MS cases (or neurological conditions in general) but also, should neither be the red flag in neurological research. Controlled studies using suitable clinical and surrogate markers (novel MRI and electrophysiological techniques) to substantiate regeneration and restoration of neurological function may provide the missing information.
Bibliography


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