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A potential cure for multiple sclerosis: the promising role mesenchymal stem cells play in the reparative process

Abstract

Background: Mesenchymal stem cells (MSCs) are multipotent stem cells derived primarily from bone marrow (BM). They exert neurotrophic and immunomodulatory effects. Understanding laboratory and patient-related factors that affect MSC biology will optimise their use as a potential multiple sclerosis (MS) treatment.

Methods: Eleven patients with relapsing forms of MS were enrolled in an ongoing Phase 1 trial, having met eligibility criteria (adult between 18 and 55, relapsing form of MS, currently on standard treatment, visual involvement, T2 hyperintense lesions on MRI). BM-derived MSCs were isolated, cultured and subsequently re-infused one time to assess tolerability. A literature review of MSC biology was conducted alongside this Phase I trial in order to examine the role of MSCs in MS therapy.

Results: Low concentration foetal bovine serum (FBS) and specific biological factors favour MSC activity. Paracrine activity yields the majority of the therapeutic effects. Faster MSC proliferation rates were observed in cells of individuals in an earlier stage of the disease. Patient age decreases proliferation capacity. The effects of gender, MS type and disease duration are less clear.

Discussion/conclusion: MSCs have great potential in MS treatment through their anti-inflammatory and reparative properties, and the trial has thus far shown that autologous MSC transplantation is safe and feasible.

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Natasha Mehandru[1]
Dr Jeffrey Cohen[2]
[1]RCSI medical student
[2]Director, Experimental Therapeutics Program, Cleveland Clinic, OH, USA

Introduction

Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease of the central nervous system characterised by neuronal demyelination and axonal loss. Autoimmune aetiology is the prevailing, though unproven, theory in which oligodendrocytes are irreversibly damaged by CD4+ T-cells, CD8+ T-cells, and macrophages.[1] Available medical therapies are limited to symptomatic relief, decreased inflammation, and decreased MRI lesion activity and relapses.[1] However, none of the approved therapies directly target oligodendrocyte repair or reverse accumulated damage and progressive forms of the disease in which degenerative processes are thought to dominate. Mesenchymal stem cells (MSCs) are currently being studied in light of the great unmet need for repair-promoting strategies.[1] MSCs are adult, multipotent, stromal-derived stem cells, which confer neuroregenerative and anti-inflammatory benefits, with potentially huge implications for reversing the damage seen in MS.[1] This paper discusses the laboratory and patient characteristics influencing MSC behaviour based on an ongoing Phase I clinical trial to assess the feasibility and safety of autologous MSC transplantation in patients with relapsing forms of MS. This research is further explored in the context of current literature, with the aim of optimising MSC activity in the ongoing trial, and with the end goal of translational medicine in relapsing MS.

Methods

Current literature regarding MSC biology was reviewed. Additionally, data from an ongoing Phase 1 clinical trial examining patient age, gender, MS type, and disease duration in relation to MSC proliferation capacity is examined.

Phase I clinical trial

The primary purpose of the ongoing Phase I trial run by Dr Jeffrey Cohen at the Mellen Center for MS Treatment and Research is to assess the feasibility, safety and tolerability of autologous, bone marrow-derived, culture-expanded MSC transplantation in patients with relapsing forms of MS. The trial is funded on behalf of the Department of Defense Peer Reviewed Medical Research Program through the National Institutes of Health. Ethical approval and written consent were obtained. Twenty-four patients were to participate; half with relapsing-remitting MS (RRMS) and half with secondary progressive or progressive-relapsing MS (SPMS). Patients with primary progressive MS were excluded due to the uninterrupted and worsening nature of this subtype, which is not typically associated with relapses. Participants were between the ages of 18 and 55, on standard therapy, and were monitored for two months pre transplantation and six months post transplantation. Inclusion criteria were: documented relapse; Expanded Disability Status Scale (EDSS) 3.0-6.5; worsening impairment; MRI lesion activity in the prior two years; and, optic nerve involvement. Only patients on FDA-approved treatment for relapsing forms of MS, or those who had failed or refused approved treatment, were included. Participants are recruited predominantly from the Cleveland Clinic. Treatment consisted of a single intravenous infusion of 1-2 x 10^6MSCs/kg. Eleven participants have been infused to date.

This ongoing trial is the first in North America to assess the activity of autologous culture-expanded MSCs in MS patients. Other trials have examined MSC and haematopoietic stem cell transplantation (HSCT) activity in the context of other pathological states in mice and human models. Allogeneic HSCTs demonstrate immunosuppressive activity through immune reconstitution of the T-cell repertoire, induced cell death of myelin-reactive T-cells, and improved functional recovery.[2] Several case series and one published phase 2a trial asserting the safety of autologous MSC infusion for secondary progressive MS at the University of Cambridge have been published.[1] Other trials assessing similar parameters are still in the recruiting phase.

Literature review

Literature regarding MSC biology and stem cell transplantation in MS was reviewed. Articles focusing on bone marrow-derived MSCs, cellular effects of MSCs, and stem cell therapy in the context of autoimmune disease were sourced.

Results

Results are divided into three categories: a literature review of MSC biology; a summary of current laboratory and patient features that affect MSC biology; and, data from the ongoing Phase 1 trial. MSC biology is first re-examined.

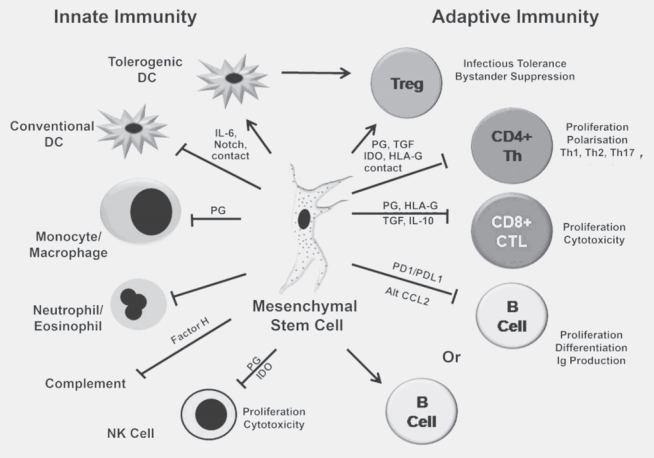

FIGURE 1: MSC role in immune response.[4]

MSC biology

MSC activity

MSCs are increasingly being examined as potential therapy for relapsing MS due to their inherent plasticity. They are attractive for transplantation as they can be readily isolated from bone marrow, rapidly expanded *in vitro*, and have a low immunogenicity.[3] A number of mechanisms have been proposed as to their method of action: transdifferentiation; cell fusion; and, paracrine activity, with the latter accounting for most of the beneficial effects. MSCs release soluble factors, which stimulate adult neural stem cells (NSCs) to differentiate into oligodendrocytes. Current research holds neurotrophic activity, in the context of appropriate microenvironment, as the prevailing method of MSC action. MSCs release neurotrophic factors, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), that promote proliferation, survival, and differentiation of NSCs; interleukins (IL-6,-7,-8,-11,-12,-14,-15) mediate haematopoiesis. Studies using rat MSC-conditioned media showed differentiation into oligodendrocytes by increased expression of oligodendrocyte markers galactocerebroside and myelin basic protein. Angiogenic, neuroprotective and synaptogenic effects were also seen. Non-neurotrophic factor (extracellular matrix proteins) secretion facilitates intercellular signalling and neurotrophic factor delivery. Through their suppression of innate and adaptive immunity, and dendritic cell function, MSCs demonstrate the potential to treat autoimmune and inflammatory diseases (**Figure 1**).[4]

MSC suppression of innate immunity

MSCs express Toll-like receptors (TLR); immune modulation is downregulated by TLR3 and TLR4 ligands, and enhanced by IFN-γ.[5] IFN-γ enhances MSC suppression of T-cell proliferation through the induction of indoleamine 2,3-dioxygenase (IDO) and prostaglandin E2.

The degree of MSC suppression is dose dependent, with high doses having an inhibitory effect.

MSC suppression of adaptive immunity

MSCs direct CD4+ T-cell differentiation towards regulatory patterns of cytokine secretion, and suppress Th1, Th2 and Th17 responses. MSCs also promote the activity of Tregs, which control alloreactive T-cell responses.[5] MSCs induce CD8+ regulatory cells and inhibit effector CD8+ cytotoxic T lymphocyte proliferation without being lysed. Influence on B-cell and macrophage activity is less understood.

Effect of MSCs on dendritic cell function

MSCs reprogram dendritic cells to become tolerogenic by converting pro-inflammatory cytokine production (IL-12, TNF-a) to anti-inflammatory cytokines (IL-10). Dendritic cells that have encountered MSCs suppress activated T-cell proliferation.[5]

Role of MSC transplantation

The significance of the neurotrophic and immunomodulatory effects discussed above relates to the role of T-cell autoreactivity and inflammation in MS-related myelin damage. Through their potent anti-inflammatory effects, MSC-derived cytokines and interleukins downregulate T-cell activity and inflammation. MSCs also stimulate adult NSCs to regenerate damaged tissue, again supporting the theory of paracrine activity. Tissue regeneration does not occur in the normal disease-repairing process, and myelin is gradually lost. Culture-expanded MSC infusion provides the resource for adult NSCs to differentiate into myelin-producing oligodendrocytes. MSC transplantation has a huge capacity for disease improvement as MSCs exist naturally in low numbers.[4]

Summary of current literature

A. Laboratory features that affect MSC biology *in vitro*

Culture conditions and media

Cryopreservation has no effect on MSC proliferation, secretion, or differentiation capacity,[6] as evidenced by no changes in colony-forming unit fibroblast (CFU-f) numbers.[7] Optimal isolation was achieved when cells were layered on Ficoll gradient, and cultured in Dulbecco's modified Eagle's medium (DMEM) with low concentration (5%) foetal bovine serum (FBS).[8]

The effects of *in vitro* factors and microenvironment on MSC activity

In vitro MSC environment influences lineage commitment; exposure to biological growth factors (bFGF, Wnt, and 2-ME DMSO) favoured differentiation into the neural lineage.[4] However, MSC transdifferentiation into neural cells does not explain the majority of MSC activity; rather, therapeutic benefits are expected to result from trophic paracrine factor secretion.

In the context of experimental autoimmune encephalomyelitis (EAE) induced in mice, NSCs and MSCs serve as bystander regulators through their neurotrophic effects.[9] MSC migration from the IV administration site to the brain has been observed, where they escape immune surveillance and in some cases differentiate into cells expressing microglial and astroglial markers.[9]

Multiple injections of neural progenitors derived from MSCs (MSC-NPs) at the onset of the chronic phase of disease resulted in improved neurological function; a single injection had no effect.[10] Reduced immune cell infiltration and demyelination, and increased endogenous nestin-positive progenitor cells were seen in EAE mice, supporting autologous MSC-NP use in MS patients.[11]

In summary, MSC neurotrophic activity prevails as the major mechanism of therapeutic benefit.

B. Patient characteristics that affect MSC biology

Effects of patient age on MSC proliferation capacity

Other studies have suggested that increased age correlates with decreased MSC proliferation capacity.[12] MSCs undergo telomere shortening at high passage numbers limiting proliferation *in vitro*, but *in vivo* constitutive expression of telomerase maintains telomeric length.[12]

Induction of neuroectodermal differentiation from MSCs was completely lost with samples from old donors.[12]

Similarly, age-related decreases in CFU-f numbers and increases in p53 and p21 positive cells, reactive oxygen species, and lipofuscin were observed.[7] Morphology changed from small spindle-like to large polygonal with successive passages.[13] Growth rates peaked in the third passage, then declined.[13] Growth factors and various cytokines gradually declined over time in ALS patient samples. MSCs at earlier passages are more suitable for stem cell therapy due to their stability, anti-inflammatory effects, and neuroprotective effects.[13] Donor age appears to decrease MSC proliferation and differentiation capacity, and anti-inflammatory effects.[13]

Effect of neurodegenerative disease on MSC function

The relative advantages of autologous versus allogeneic MSC transplantation are widely debated with no definitive evidence asserting one's superiority to the other. Larghero *et al.* support autologous MSC transplantation after showing that BM-MSCs from systemic sclerosis patients *in vivo* demonstrate the same proliferative and immunosuppressive properties as their healthy counterparts.[14] However, Koh *et al.* indicate that pluripotency and trophic factor secretion capacity of BM-MSCs from amyotrophic lateral sclerosis (ALS) patients are reduced in proportion to a poorer prognosis.[15] Autologous MSCs from ALS patients demonstrated reduced migration when compared to healthy donors.[16] Expression of B-PIX-an intracellular factor implicated in migration was significantly reduced in ALS-MSCs. Restoring expression via genetic manipulation restored migration ability, asserting the potential role of genetic manipulation in autologous MSC transplantation.[16]

MSCs in other contexts

MSCs have been transplanted in conjunction with allogeneic HSCTs to enhance engraftment, and as prophylaxis against graft-versus-host disease.[17]

As MSC therapy is still young in its therapeutic evolution, ongoing trials such as this one focus on utilising the ability of these multipotent mesenchymal cells to secrete neurotrophic factors and mediate immune effects as a source of MS therapy.

Table 1: Patient characteristics and MSC cell yield.

Patient	Age	Gender	MS type	Years since onset of symptoms	Days in in culture	Passage # at harvest	Final cell yield (x10^8)	% of MSCs marker positive CD105/CD73	CD45/CD14
1	46	M	SPMS	11	34	2	5.86	96.94	0.04
2	40	F	RRMS	10	21	1	1.94	98.73	1.15
3	45	F	SPMS	10	41	3	1.375	95.79	0.28
4	56	F	SPMS	36	34	3	1.1	98.80	0.07
5	42	F	SPMS	22	39	3	0.558	95.51	0.82
6	43	M	SPMS	5	24	2	2.5	97.41	0.08
7	40	F	SPMS	18	18	1	4.75	98.31	0.76
8	43	F	RRMS	5	20	1	5.02	98.93	0.51
9	47	M	RRMS	4	20	1	2.63	98.07	0.64
10	55	M	SPMS	10	34	3	2.31	98.25	0.17
11	47	F	RRMS	21	16	1	3.46	95.24	0.46

SPMS = secondary progressive multiple sclerosis. RRMS = relapsing remitting multiple sclerosis. MSC = mesenchymal stem cell.

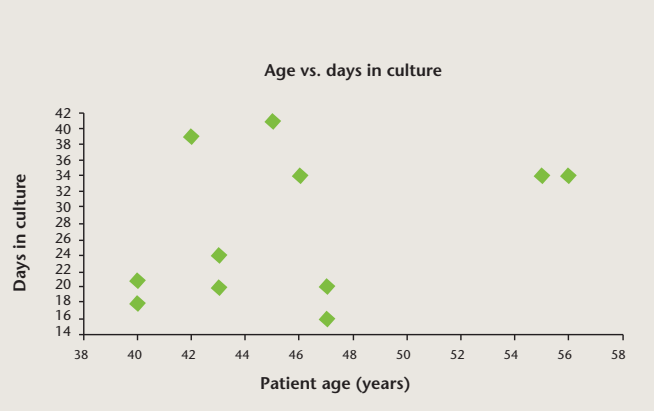

FIGURE 2: Effect of each participant's age on the time taken for MSCs grown in vitro *to achieve target dose for infusion.*

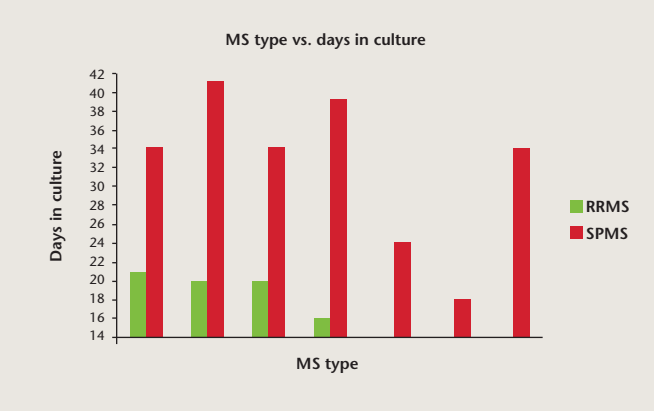

FIGURE 3: Effect of MS type on the proliferation rate of MSCs as measured by the number of days in culture to achieve target infusion dose. Each bar represents a single patient. SPMS = secondary progressive MS. RRMS = relapsing remitting MS.

Summary of patient characteristics and MSC laboratory cell culture results

A summary of data obtained for each participant who received an infusion of autologous MSCs in the Phase I trial is shown in **Table 1**. Duration of *in vitro* growth and final cell yield for MSCs is discussed in the context of age, gender, MS type and duration. The ongoing Phase I trial roughly supports current literature asserting that increased age correlates with decreased proliferative capacity, as evidenced by increased length of culture necessary to reach the target dosage (**Figure 2**). The target infusion dose is up to 2 x 10^6 cells per kg. When feasible, the number of passages was limited to the least number possible to allow for the most stable MSCs. Culture duration was shorter in RRMS vs. SPMS, suggesting a faster proliferation rate earlier in the disease (**Figure 3**). Most patients with MS begin with an RR course, but subsequently evolve into an SP course after about 10-15 years. Disease duration is thought to decrease overall proliferation capacity, though this was not apparent in the trial (**Figure 4**). Current literature is unclear about the qualitative differences of MSCs derived from patients with autoimmune disease versus those from healthy counterparts.

Discussion

Our understanding of MSC activity, although not complete, points to a dominant role in the anti-inflammatory and reparative process, primarily through neurotrophic effects. MSCs are feasible transplant options due to their ability to be readily isolated and expanded. MSCs secrete a range of factors involved in the differentiation of adult NSCs and neural tissue regeneration, although the quality of the regenerated tissue and extent of repair is largely unknown. Laboratory features such as low concentration FBS, FRP surfaces with FGF2, biological factors, microenvironment, and genetic manipulation of receptor-ligand interaction favour activity, while cryopreservation and disease time point seem to have little effect. Furthermore, patient age decreases MSC proliferation capacity and anti-inflammatory effects. Limited data from the ongoing trial limits a conclusion about the effects of gender, MS type, and disease duration on proliferation

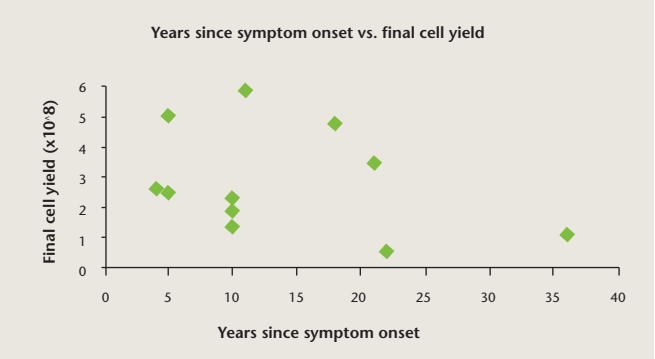

FIGURE 4: The number of years since the onset of the first MS symptom compared to the final MSC yield.

capacity. Most patients with MS begin with an RR course then evolve into an SP course after 10-15 years. Therefore, it is difficult to distinguish between the effects of age, disease duration, and disease course on MSC proliferation rate in culture and eventual yield. Furthermore, as the trial is ongoing, limited data makes it difficult to identify clear patterns between patient characteristics and disease course. However, taken together, these data suggest a connection between disease course and MSC behaviour in culture – namely, slowed proliferative capacity in MSCs from patients with SPMS compared to RRMS.

Nonetheless, the ongoing trial achieves its primary goal of asserting that autologous MSC transplantation in MS patients is safe and feasible, as no adverse events have yet been reported. As the trial is still early in its progress, comments cannot be made on long-term effects, defined as after six months post infusion. Nevertheless, it is important to note that there have been no immediate adverse effects relating to the infusion of autologous MSCs. Confirming such tolerability will allow for the next step of MSC therapy development, which is to carry out Phase II trials assessing reparative effects and efficacy.

Conclusion

MSC therapy offers a novel way to approach the treatment of neurodegenerative diseases. MSCs have promising advantages over both current medical therapy and other stem cell sources. The next step is to solidify our understanding of MSC activity and cultivate optimal MSCs capable of going one step beyond halting disease damage and ultimately reversing the disease process, providing a potential cure for MS.

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