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The potential use of adult stem cells for the treatment of multiple sclerosis and other neurodegenerative disorders

Shimon Slavin [a,*], Basan G.S. Kurkalli [a], Dimitrios Karussis [b]

[a] *International Center for Cell Therapy & Cancer (ICTC), Tel Aviv Medical Center, 6 Weizman Street, Tel Aviv 64239, Israel*

[b] *Department of Neurology and the Agnes Ginges Center for Neurogenetics, Hadassah Hebrew University Hospital, Jerusalem 91120, Israel*

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Abstract

No specific treatment exists for patients with multiple sclerosis (MS) who fail to respond to conventional immunosuppressive and immunomodulating modalities. Furthermore, no method is available for regeneration of existing defect in the central nervous system (CNS). The ultimate goals of MS treatment, similarly to other autoimmune diseases, are twofold: first, to eliminate self-reactive lymphocytes and to prevent de novo development of self-reactivity by induction of self-tolerance. Second, attempting regeneration and repair of existing damage. In the case of MS, there is a need to stop the ongoing process of inflammation against the CNS by self-reactive lymphocytes thus facilitating spontaneous re-myelinization while in parallel attempt to recover existing neurological deficits caused by the autoimmune process resulting in demyelinization. Cell therapy stands out as the most rationale approach for neurological regeneration. In the absence of clinically applicable approaches involving the use of embryonic stem cells, we are investigating the feasibility and efficacy of enriched autologous mesenchymal stromal cells (MSC) injected intrathecally and intravenously to induce in situ immunomodulation and neuroprotection and possibly facilitate repair of the CNS in patients with MS and other neurodegenerative disorders. Our preclinical results suggest that bone marrow cells may provide a source of stem cells with a potential for migration into inflamed CNS and differentiate into cells expressing neuronal and glial cell markers. Based on the preclinical data, we are currently evaluating the safety of a similar therapeutic approach in a small group of patients with MS and other neurodegenerative diseases.

Keywords: Stem cells; Mesenchymal stromal cells (MSC); Experimental autoimmune encephalomyelitis (EAE); Multiple sclerosis (MS); Neurodegeneration; Neurological repair; Immunomodulation; Central nervous system (CNS)

1. Introduction

Available methods for the treatment of multiple sclerosis (MS) are only partially effective, due to inadequate control of self-reactive lymphocytes in one hand and ineffective re-myelinating regenerating mechanisms, which results in cumulative disability and irreversible axonal/neuronal damage, on the other [1,2]. Innovative approaches are urgently required for better control of anti-self reactivity and facilitation of neurological repair. In the past, we have introduced the concept of lymphoablative treatment for elimination of all lymphocytes, self-reactive lymphocytes included, followed by transplantation of autologous stem cells, preferably enriched or depleted of self-reactive T cells, towards re-induction of self-tolerance [3–6]. Ongoing clinical investigations suggest that selected patients with malignant MS fully resistant to all available modalities may indeed respond positively to autologous stem cell transplantation [7–15]. Ideally, simpler approaches are desirable for immune regulation of the ongoing anti-central nervous system (CNS) inflammatory process. Moreover, in the absence of clinically available options for regeneration of existing damage, and lack of ability to use embryonic or neural stem cells, newer therapeutic interventions which may offer effective neuroprotection and neurological repair are required.

Adult bone marrow cells may be applicable for meeting some of these goals. First, safe elimination of lymphocytes by lymphoablative conditioning, followed by transplantation of T cell depleted stem cells may provide a partial answer to the

* Corresponding author. Tel.: +972 52 426 2490.
E-mail address: slavinMD@gmail.com (S. Slavin).

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first treatment goal, since successful elimination of lymphocytes and de novo regeneration of the T cell repertoire is likely to result in re-induction of self-tolerance due to anticipated apoptosis of self-reactive T cells in *status nascendi* in the thymus, similarly to the process that results in self-tolerance in utero [3]. Much more uncertainty exists as to the potential of adult stem cells for repair of existing neurological deficits in patients with MS as well as other degenerative central nervous system disorders. Until recently, it was considered that no repair mechanisms exist in the central nervous system. More recently, experimental data suggest that neuronal stem cells may exist in certain areas of the CNS, suggesting that in principle, under proper conditions and elimination of the cause of the disease to start with, such cells may be activated to induce repair of neurological deficits.

Following proof of principle in preclinical studies using the animal model of MS, EAE, our ongoing investigations focus on the potential use of bone marrow-derived mesenchymal stem cells (MSC) for in situ immune regulation and possibly induction of neurological repair and re-myelinization in an ongoing preliminary clinical trial.

2. Preclinical support of the working hypothesis

The therapeutic potential of bone marrow-derived MSC was documented in the chronic progressive model of EAE induced in C57BL/6 mice with the MOG 35-55 peptide. Isolation of bone marrow-derived MSCs was accomplished by culturing bone marrow cells obtained from syngeneic femora. Enrichment for MSC was documented 2 weeks later by positive staining of cultured cells with anti-CD29 and CD44 and negative staining for CD45 (hematopoietic lineage marker) as was also documented earlier [16–18]. MSC cultured under these conditions appeared with thin and long processes resembling neural-like and glial-like cells. Cells featured positive immunostaining for neural-lineage cell markers: nestin (neural marker), tubulin beta-III (neural marker), GFAP (astrocyte marker) and O4 (oligodendrocyte marker). In the model of chronic EAE induced by the MOG 35-55 peptide, MSCs showed a strong migratory potential to white matter lesions, in correlation to the site and degree of inflammation. The clinical course of EAE was significantly ameliorated in animals treated with purified MSCs, following both intracavitary and intravenous MSC administration (20 and manuscript submitted for publication). Histopathological analysis of CNS sections in these successfully treated animals revealed a strong neuroprotective effect induced by MSC transplantation.

To investigate the immunomodulatory effects of purified MSCs, myelin-sensitized lymphocytes (obtained from EAE mice), were cultured with different MSCs concentrations in the presence of MOG peptide or of the mitogen concanavalin A. MSC induced a strong suppression of specific and non-specific proliferative responses of T lymphocytes, thus suggesting that immune regulation of self-reactivity may also occur in vivo.

Considering the fact that our preclinical results demonstrated that MSC could result in immune regulation and neuroregeneration in the model of chronic EAE [40–41], it seemed reasonable to consider a similar therapeutic approach in the management of neuroimmunological and neurodegenerative diseases such as MS and amyotrophic lateral sclerosis (ALS), respectively, in consenting patients with progressive disease failing to respond to any of the available modalities.

3. Pilot clinical trials to investigate the safety and feasibility of intrathecal treatment with MSC in MS and ALS

Based on the aforementioned rationale and supported by data in preclinical animal models as indicated above, and considering the fact that no alternative effective treatment exists for patients with existing neurological deficits resulting from active MS and ALS, we decided to conduct a pilot clinical trial and treat patients in need with no other treatment options available, starting with ALS, to prove that treatment with MSC is feasible and safe, as a preliminary study to be followed in patients with MS, where in addition to neurological repair, such a procedure may be also indicated for regulation of self-reactive lymphocytes. After approval of Institutional Review Board, autologous bone marrow-derived cells were cultured under strict sterile conditions in clean rooms. MSC were enriched within 3–4 weeks. Autologous MSC were injected intrathecally and intravenously to 12 patients [not all but few of the patients were injected both intrathecally and intravenously with MS and other neurological diseases like amytorphic lateral sclerosis (ALS), cerebral atrophy (CA), motor neuron disease (MND) and progressive spinal palsy (PSP)] after confirmation of sterility of the cells with 2-week culturing system by the Department of Microbiology of the Hadassah Medical Center. Whereas it is too early to report the outcome of this experimental treatment with MSC, suffice is to mention that some patients claim for demonstrable benefit, but objective evaluation must be carried out in order to separate any possible placebo effects from wishful thinking. The safely of intrathecal and intravenous infusion of MSC to both patients with ALS, MS and a few other with different indications for tissue repair, like spinal injury, were well documented, until now.

4. Discussion

Based on preclinical data and the rationale to use cell therapy for down-regulation of anti-self reactivity on the one hand, with the goal in mind to enable spontaneous re-myelinization and possibly neurological repair of existing damage, our preliminary study indicated that intrathecal and

intravenous autologous MSC therapy is feasible and safe. Based on the murine data we hope that intrathecal injection of MSC will maximize the chance of homing of such cells to desirable locations in the central nervous system. All treated patients are under observation to exclude late side effects, which until now were not observed.

Only one patients (ALS) developed self-limited mild meningeal signs starting immediately after intrathecal cell injection, with no evidence of any infective agent. All manifestations disappeared within 2 days, most likely due to residual DMSO injected with the cells due to inadequate washing of thawed cryopreserved MSC by a new technician. No other side effects were noticed within an observation period of >1 year.

The use of marrow stromal cells as stem cells for non-hematopoietic tissues is already seriously considered for a large variety of indications [19–21]. Likewise the immunoregulatory role of MSC was also documented by several investigators in different model systems and on different lymphocyte subsets in mice and man [22–29]. In addition, transition of bone marrow-derived MSC to cells that express neural markers was also previously demonstrated in vivo and in vitro using murine and human bone marrow-derived cells, respectively [30–34]. The use of MSC for treatment of experimental autoimmune encephalomyelitis inducing amelioration of the signs of disease, most likely through induction of T-cell anergy was also previously reported [20,35]. In view of the multipotentiality of MSC, in parallel with attempts to regenerate the CNS by MSC, other investigators are trying to use bone marrow-derived MSC for treatment of other disorders that may be successfully treated by autologous cell therapy [36–39].

Based on the limited experience, it seems that further trials utilizing MSC derived from adult stem cells may be warranted, preferably in patients at an earlier stage of the disease, to allow potential neurological regeneration before irreversible changes occur in the CNS. Future culturing of bone marrow-derived adult multipotential MSC, possibly with additional biological agents to further enhance neurogenesis on the one hand and/or to rejuvenate degenerating neurons are currently under investigation. Evaluation of the potential clinical benefits of autologous MSC treatment requires further investigations in larger cohorts of patients with MS and other neurological diseases, in prospective randomized clinical trials, which may be fully justified, especially since the procedure can be technically successfully accomplished with no major risks, based on our preliminary study.

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