Stem cell transplantation in multiple sclerosis

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Purpose of review
The recent advances in our understanding of stem cell biology, the availability of innovative techniques that allow large-scale acquisition of stem cells, and the increasing pressure from the multiple sclerosis (MS) patient community seeking tissue repair strategies have launched stem cell treatments as one of the most exciting and difficult challenges in the MS field. Here, we provide an overview of the current status of stem cell research in MS focusing on secured actuality, reasonable hopes and unrealistic myths.

Recent findings
Results obtained from small clinical studies with transplantation of autologous hematopoietic stem cells have demonstrated that this procedure is feasible and possibly effective in severe forms of MS but tackles exclusively inflammation without affecting tissue regeneration. Results from preclinical studies with other adult stem cells such as mesenchymal stem cells and neural precursor cells have shown that they may be a powerful tool to regulate pathogenic immune response and foster tissue repair through bystander mechanisms with limited cell replacement. However, the clinical translation of these results still requires careful evaluation.

Conclusion
Current experimental evidence suggests that the sound clinical exploitation of stem cells for MS may lead to novel strategies aimed at blocking uncontrolled inflammation, protecting neurons and promoting remyelination but not at restoring the chronically deranged neural network responsible for irreversible disability typical of the late phase of MS.

Keywords
autoimmunity, experimental autoimmune encephalomyelitis, multiple sclerosis, stem cells, transplantation

Introduction

In the last few years, the extraordinary progress in our understanding of adult stem cell biology has led to major advances in the field of cell therapy, allowing us to translate our basic knowledge about different kinds of stem cells into therapeutic strategies aimed at treating neurological diseases such as multiple sclerosis (MS). Although autologous hematopoietic stem cell transplantation (AHSCT) has now been proven to be a powerful, although risky, therapy for some forms of MS, other stem cell types have gained attention as potential future therapeutic options for MS. However, experimental data have posed us with an unforeseen scenario. As most scientists moved into the stem cell arena due to an unmet need for therapies for tissue repair, current evidence suggests that stem cells that have been proven to ameliorate symptoms and protect neural cells in experimental autoimmune encephalomyelitis (EAE), a model of MS, also have a limited, if any, capacity for transdifferentiating into neural cells, but may foster tissue protection and repair through unexpected mechanisms of action.

Autologous hematopoietic stem cells for the treatment of autoimmunity

AHSCT has been proposed for severe autoimmune disorders unresponsive to conventional treatments [1] based on results from experimental models [2]. AHSCT procedure consists of mobilization from the bone marrow of peripheral blood stem cells (PBSCs) usually with cyclophosphamide in combination with granulocyte-colony stimulating factor. PBSCs expressing the surface antigen CD34 are collected by leukapheresis and cryopreserved. The graft can be manipulated with a positive selection of $CD34^+$ cells or a negative depletion of T cells, in order to eliminate autoreactive clones. The patient is then treated with the conditioning regimen, usually with high-dose cytotoxic agents such as BEAM (BCNU, carmustine, etoposide, cytosine–arabinoside and melphalan), total body irradiation (TBI) or other various combinations of

cytotoxic agents. The conditioning regimens are usually classified as high-intensity (TBI or any busulphan-containing protocol), medium-intensity (BEAM, carmustine and cyclophosphamide) or low-intensity regimens (cyclophosphamide alone or fludarabine-based schemes). The cryopreserved graft is then re-infused into the patient and antithymocyte globulin (ATG) is administered in order to eradicate self-reactive T cells. After a period of aplasia of 2–3 weeks, engraftment occurs. The rationale of the procedure relies on intense immunosuppression aimed at destroying autoreactive cells and the subsequent immune reconstitution that is associated with profound qualitative changes of the immune repertoire.

Autologous hematopoietic stem cell transplantation in multiple sclerosis: clinical outcome

More than 400 MS cases have been reported so far in the European Bone Marrow Transplantation database. However, although no phase III clinical trial has been completed yet, a series [3••] of small phase I/II studies have been reported. Despite the concerns regarding different protocols and disease forms treated, 60–70% of patients after 3 years and 50–60% after 6–8 years do not progress from transplantation [4]. In a recent study [5], 50 MS patients were treated with BEAM and ATG followed by AHSCT at different disease phases with Expanded Disability Status Scale (EDSS) ranging from 1.5 ('early AHSCT') to 8.0 ('salvage AHSCT'). The procedure was well tolerated and effective and 62% of patients improved at least 0.5 points on EDSS, particularly when AHSCT was performed in young individuals. Progression-free survival at 6 years was 72%. The Canadian MS BMT Study Group [6] treated 17 aggressive MS patients with a high-intensity conditioning regimen (busulphan and cyclophosphamide) with ex-vivo and in-vivo T-cell depletion. These patients had a favorable outcome, with 75% progression-free survival at 3 years, without any relapse or new MRI lesions nearly 5 years after treatment [6]. The retrospective analysis of transplanted patients data performed in 2002 [7] and 2006 [4], did not show any difference in disability progression between high and intermediate-intensity regimens, whereas a correlation was observed for transplant-related mortality (TRM; 6 and 5.3%, respectively) and regimens including busulphan. Although TRM has been reported to decrease to 1.3% in a recent analysis [3••], most likely as a result of better patient selection and improved experience of the transplanting centers, low-intensity treatments, with minimal myelotoxic effects, have been proposed [8•]. In a recent study, 21 young, relapsing–remitting MS (RRMS) patients with mild disability and short disease duration were treated using a low-intensity conditioning regimen (cyclophosphamide 200 mg/kg followed by alemtuzumab or ATG). After 3 years, 81%

of patients improved by at least 1 point on EDSS and 62% were disease free. Modest toxicity was reported and 23% of patients relapsed after 6–16 months. Recently, in an open-label study [9], the effect of low-intensity (cyclophosphamide and rabbit ATG) and medium-intensity (BEAM and horse ATG) regimens was addressed. Regardless of a similar clinical outcome, individuals treated with cyclophosphamide and rabbit ATG displayed significantly less toxicity as compared with those treated with BEAM and ATG.

AHSCT has also been reported to have a significantly positive impact on rapidly evolving, 'malignant' MS refractory to conventional treatments [10]. In a small cohort of young patients with RRMS presenting with high number of relapses per year and high EDSS, AHSCT was able to halt disease progression and reverse disability [11]. Overall, these studies confirm that AHSCT is more effective in very active, young RRMS individuals with a short disease history.

Autologous hematopoietic stem cell transplantation-related changes of the immune repertoire

The restoration of immune tolerance following AHSCT is characterized by a profound renewal of the T-cell repertoire mainly due to the expansion of naive $CD4^+$ T cells of recent thymic origin [12]. This study [12] suggests for the first time that AHSCT results in the induction of a new immune system less prone to self-reactivity. Although self-reactive T cells may persist after transplantation [13,14], they do not seem to arise from mobilized HSC-enriched graft [15•]. Thus, some peripheral or central nervous system (CNS) infiltrating T and B-cell clones may survive the conditioning regimen, as demonstrated by the persistence of oligoclonal bands in the cerebrospinal fluid of most patients and high levels of soluble CD27, a marker of lymphocyte activation, after AHSCT [16•].

Future perspective for autologous hematopoietic stem cell transplantation in multiple sclerosis

At the present time, a few studies on AHSCT in severe forms of MS are ongoing, including ASTIMS (Autologous Stem cell Transplantation International Multiple Sclerosis), a European Union-based phase II study comparing the effect of AHSCT versus mitoxantrone, which has been recently stopped for the insufficient accrual of patients, the Halt-MS study, a US trial, investigating the effect of BEAM, ATG and $CD34^+$ cell selection in RRMS or progressive–relapsing MS patients and the 'Stem Cell Therapy for Patients with MS Failing Interferon' randomized clinical trial in the United States

enrolling inflammatory patients with the aim of comparing transplantation of unmanipulated autologous PBSCs using a conditioning regimen of cyclophosphamide and ATG versus US Food and Drug Administration-approved MS therapies. The scientific community interested in AHSCT for MS met recently in Florence on 19–20 November 2009, discussing the possible design of a two-arm study focusing on young rapidly deteriorating patients refractory to standard therapies and with clinical and MRI signs of disease activity. Patients will be randomized to an intermediate intensity regimen or the best available treatment, with the possibility to crossover into the other study arm in case of continuing disease activity.

Although the clinical effectiveness of AHSCT compared with conventional therapies is still debated, a recent analysis [17] of cost effectiveness of AHSCT versus mitoxantrone in secondary progressive MS suggests that the probability of AHSCT being cost effective, when TRM is low, depends on the achievement of a long enough disease stabilization (10 years).

Mesenchymal stem cells definition

Multipotential stromal precursor cells were first isolated from the bone marrow, as the common ancestors of mesenchymal tissues such as cartilage, fat, bone and other connective tissues [18], and commonly termed as mesenchymal stem cells (MSCs). Many other tissues have been reported to be the source of MSCs, more recently the vasculature being a source of perivascular cells with the phenotype of MSCs [19,20•]. The study [19] demonstrates that bone marrow stem cells capable of giving rise to the complete hematopoietic microenvironment reside exclusively in a small fraction of perivascular cells. However, such a conventional view of marrow stromal cell plasticity was challenged by several studies reporting their capability to also differentiate into cells from unrelated germ lineages including neural cells [21,22]. This heterogeneity is reflected by a complex transcriptome encoding a wide array of proteins involved in a large number of diverse biological processes that are likely to result in some unexpected therapeutic features [23].

Mesenchymal stem cells display immunomodulatory properties

Several reports have demonstrated in the last few years that MSCs are endowed with a robust regulatory effect on many cells of innate and adaptive immunity [24••]. MSCs were first demonstrated to inhibit in-vitro proliferation of T cells [25,26] and this was later demonstrated to be the result of an inhibition of T-cell division [27]. More recently, it has become clear that the immunoregulatory features of MSCs are elicited by inflammatory cytokines, mainly interferon-gamma and tumor necrosis factor-

alpha, resulting in the production of species-specific immunosuppressive factors, namely indoleamine 2,3 dioxygenase in humans and nitric oxide in mice [28]. The in-vivo translation of these results were achieved when the intravenous (i.v.) injection of MSCs into EAE mice led to the striking inhibition of proliferation of ex-vivo isolated lymph node T cells [29].

B lymphocytes are also the target of MSCs immunosuppressive activity. In fact, MSCs can inhibit in-vitro proliferation of B cells, differentiation to plasma cells and production of antibodies [30–32]. Similarly to what was observed for T cells, i.v. MSCs administration in EAE-affected mice resulted in the inhibition of the production of immunoglobulins specific for the encephalitogenic myelin antigen proteolipid protein [33]. Interestingly, the suppression of immunoglobulin production was recently demonstrated to depend on the effect of a variant of the MSC-derived chemokine (C–C motif) ligand 2 (CCL2), which is proteolytically degraded by matrix metalloproteinases secreted by MSCs themselves [34••].

A third cell type significantly affected by the interaction with MSCs is the dendritic cell. MSCs can spoil dendritic cell in-vitro maturation resulting in an impaired secretion of interleukin (IL)-12 [35] and increased production of IL-10 [36]. MSC-induced immature dendritic cells do not upregulate major histocompatibility complex and costimulatory molecules and poorly present antigens to naive T cells [37]. These findings suggest that the net effect of MSCs on adaptive immunity is the consequence of a direct inhibition on T and B lymphocytes but also of an impaired ability of MSC-affected immature dendritic cells to properly instruct T cells, which, in turn, could also affect the capacity of T cells to provide help to B cells [24••].

Are mesenchymal stem cells neuroprotective?

The original observation that MSCs can transdifferentiate into neurons [21,22] *in vitro* and, upon in-vivo administration, acquire some markers of neural cells [38] is currently a matter of controversy, as the marker analysis alone may well be due to an aberrant expression [39,40]. Since then, in-vitro MSC neuronal differentiation has been achieved by treatment with trophic factors [41] and also by genetic manipulation [42]. Although the exploitation of 'in-vitro neuralized' MSCs appears a promising strategy for the treatment of neurodegenerative diseases, it is not known whether in-vitro transdifferentiation would result in the loss of other therapeutic properties such as immunoregulatory features, thus hampering their use in MS. On the contrary, current evidence from EAE suggest that in-vivo administration of in-vitro expanded undifferentiated MSCs does not result in a substantial

CNS engraftment and acquisition of a neural phenotype [33,34••,43,44••,45]. Regardless of the limited evidence of transdifferentiation by histological analysis, there is no clear experimental confirmation that MSC-derived neuronal cells are able, when transplanted *in vivo*, to correctly integrate among neural networks as functional neurons. However, MSCs could act on neural cells through other modalities that may lead to tissue repair. For example, MSCs have been demonstrated *in vitro* to rescue neurons from apoptosis [46,47•] and promote neurite outgrowth [48]. It has also been demonstrated that MSCs are able to produce a wide variety of trophic factors, cytokines, chemokines and antioxidant molecules, resulting in increased neuronal survival [34••,49,50•,51]. Moreover, some secreted proteins could trigger host brain plasticity, thereby inducing endogenous precursor proliferation that, in turn, may lead to neurogenesis [52] and oligodendrogenesis [44••,53,54].

Administration of mesenchymal stem cells improves experimental autoimmune encephalomyelitis

Clinical interest in EAE was sparked by the hope that MSCs could, on the one hand halt the autoimmune attack on the CNS and, on the other hand, repair injured tissue. Preclinical studies demonstrated that this hypothesis was correct but also that MSCs were clinically effective when cells were given early, before the onset of the chronic phase of disease, sustained by irreversible damage of the nervous system. Unexpectedly, pioneer experimental work demonstrated that a striking clinical effect was achieved in EAE by i.v. administration of either syngeneic (mouse) [29] or xenogeneic (human) [55] MSCs. In fact, i.v. administration resulted in the induction of peripheral immune tolerance leading to the inhibition of pathogenic T and B-cell reactivity [29,33]. Many other groups have now confirmed that MSCs can ameliorate EAE in different animal models when injected intravenously [43,44••,45], intraventricularly [56] and even intraperitoneally [34••]. Although no clear evidence of neural transdifferentiation was obtained in most of these studies [33,34••,43,44••], MSCs administration was sufficient to decrease axonal loss and improve neuronal survival [33,56,57], as well as to induce oligodendrocytes proliferation and remyelination [44••]. These findings support the concept that MSCs are likely to foster CNS repair, acting as tolerogenic cells, elicited by inflammatory cues, on autoimmune cells and as bioactive providers of trophic and antiapoptotic factors leading to neuroprotection [51,58].

Clinical experience with mesenchymal stem cells in multiple sclerosis

MSCs have been utilized in a few studies with limited numbers of patients and also as single-case, uncontrolled treatment by many patients obtaining yet unproven stem cell therapies, a phenomenon known as 'stem cell tourism' [59]. Despite the fact that allogeneic MSCs have been shown to be well tolerated and effective in treating graft versus host disease (GVHD) [60••], autologous MSCs from MS individuals share almost identical functional properties with those from healthy individuals [61] and, therefore, have been preferred thus far for clinical exploitation in MS. In pioneer studies, the administration of autologous MSCs, either i.v. or intrathecal, was well tolerated and, despite the lack of a proper clinical design to address efficacy, exhibited some beneficial effect on clinical and MRI parameters [62,63•,64]. In order to avoid the proliferation of numerous small studies utilizing MSCs for the treatment of MS, a consensus [65••] on their utilization was recently published by a panel of experts, setting the stage for an international phase II clinical trial. The consensus recognized that, at this stage, current evidence supports the i.v. administration of autologous MSCs as inhibitors of the autoimmune response in patients continuing to show inflammatory activity despite attempts to treat with immunomodulatory agents, and proof of principle of MSC biological activity on validated parameters such as MRI metrics should be achieved before testing their ability to promote tissue repair.

Neural stem cells definition

Neural precursor/stem cells (NPCs) can be detected in the developing and adult CNS as a heterogeneous population of proliferating, self-renewing and multipotent cells, with the ability to differentiate toward different neuroectodermal cell lineages [66,67]. In the study [67], the authors describe that the therapeutic features of NPCs were based mainly on bystander mechanisms. In the adult CNS, at least two distinct areas, the subventricular zone of the lateral ventricles and the subgranular zone of the hippocampal dentate gyrus, have been demonstrated to contain multipotent progenitors of neural cells and, therefore, have been named CNS germinal neurogenic niches [68]. Within the neurogenic niches, NPCs are a restricted and diverse population of progenitors whose behavior is regulated by a specialized microenvironment leading to the generation of different types of neurons [69]. It has been demonstrated that endogenous NPCs residing in the germinal niches are mobilized to demyelinated periventricular lesions by inflammatory cues occurring during EAE and proliferate, giving rise to neural cells [70]. Similarly, it has been shown that activation of early glial precursors from germinal niches occurs in MS, wherein they could give rise to oligodendrocyte precursors [71]. However, the intrinsic CNS ability of undergoing self-repair is impaired during MS due to microenvironmental cues [72••] that could be directly dependent on molecules associated with

inflammation [67], due to a dysregulation of embryoge-netic pathways [73], or both.

## Therapeutic plasticity of neural precursor/stem cells

Although NPCs are the natural progenitors of neural cells, and NPCs-based therapies have been fairly regarded as a source for newly formed CNS cells [74], recent experimental data have shown that they display unexpected therapeutic plasticity, which mostly relies on diverse bystander effects [67]. A seminal study [75] demonstrated that i.v. or intraventricular administration of NPCs in mice with EAE led to their engraftment into demyelinating lesions and to some level of differentiation into nervous cells, including oligodendrocyte progenitors actively remyelinating axons. Despite this early report, most studies have shown very low neural differentiation of transplanted NPCs. Conversely, it was reported that systemically injected NPCs ameliorate EAE through anti-inflammatory and neuroprotective mechanisms [76,77]. These bystander mechanisms occur through the engraftment of i.v. transplanted NPCs in the peri-vascular area of inflamed CNS vessels where they form atypical ectopic niches and release neurotrophins, immu-nomodulatory molecules and factors inhibiting the for-mation of glial scar [67]. Recent evidence shows that i.v.

injected NPCs also display regulatory functions of the immune response within peripheral lymphoid organs through the inhibition of myelin-specific peripheral T cells [78] and an impairment of dendritic cell functions through a bone morphogenetic protein 4-dependent mechanism [79]. The study [78] shows that NPCs display also the ability to regulate autoreactive immune cells in the peripheral blood. Moreover, a recent study [80•] has shown that intraventricularly transplanted NPCs could lead also to the induction of endogenous neurogenesis, as demonstrated by a mitogenic effect on host oligodendro-cyte precursors. Although the clinical translation of these preclinical studies is under scrutiny, it has been demon-strated that human NPCs can be safely administered intravenously in nonhuman primates with EAE and result in the successful amelioration of symptoms and disease mainly through immunoregulatory mechanisms [81•].

Other (stem) cells for the treatment of multiple sclerosis

Other types of myelin-forming cells have been trans-planted into rodents affected by experimental CNS demyelination [72••]. For example, transplantation of oligodendrocyte progenitor cells into demyelinated lesions inside the spinal cord leads to extensive remyelination

Figure 1 The mechanisms involved in the therapeutic plasticity of adult stem cells for multiple sclerosis are depicted
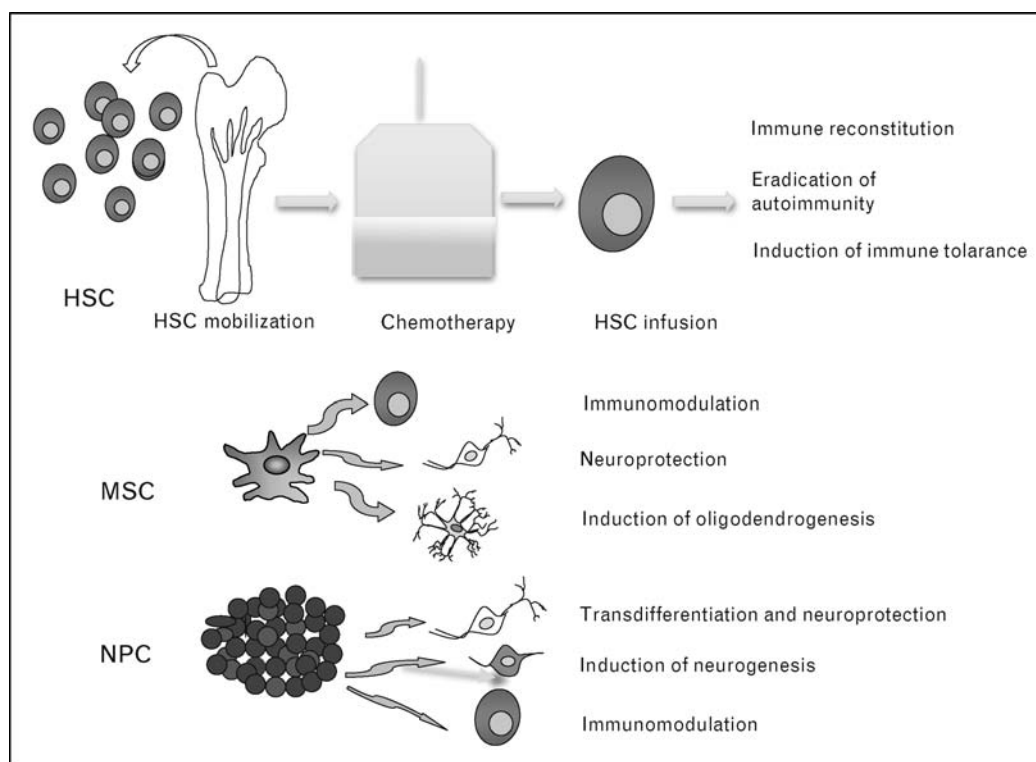
HSC, hematopoietic stem cell; MSC, mesenchymal stem cell; NPC, neural precursor cell.

[82]. Similar results have been obtained following the transplantation of Schwann cells [83], olfactory ensheathing cells [84] and also embryonic stem cells (ESCs) [85]. Interestingly, it has recently been shown that in-vitro differentiation of ESCs to multipotent neural progenitors ameliorates EAE but results in the loss of their capacity of remyelinate upon in-vivo transplantation [86]. Several concerns arise from these approaches. In particular, lineage-restricted myelinogenic cells show limited growth and expansion characteristics *in vitro* and, following in-vivo transplantation, induce scarce remyelination, often due to environmental cues limiting precursor differentiation and proliferation and their limited ability to spread far from the transplantation site [72**]. Further, the use of ESCs is restricted by ethical and technical concerns about source of cells and the intrinsic risk of tumor formation. On the basis of these considerations, such strategies require further studies before their clinical exploitation for the treatment of MS.

Conclusion

To date, the only 'stem cells' that can be considered a therapeutic option for MS are AHSCs, whose administration, however, must be mainly considered as a rescue therapy following intense immune suppression with cytotoxic drugs and may, at best, lead to an immune system less prone to autoimmunity (Fig. 1). Thus, in this case, 'stemness' *per se* does not represent a therapeutic opportunity for CNS repair. Other adult stem cells are likely to provide a realistic opportunity for remyelination and axon reorganization due to their therapeutic plasticity. It is noteworthy that results from the administration of adult stem cells in preclinical models of MS moved from almost opposite starting points to end up with some common therapeutic features, although occurring through complex and different mechanisms of action. In fact, NPCs were first described as cells giving rise to newly formed neural cells capable of remyelinating [75], then were shown to provide pleiotropic neuroprotective factors *in situ* [77] and, more recently, to also display a regulatory effect on the autoimmune response [78] and induce endogenous neurogenesis [80*]. In contrast, MSCs were first demonstrated to induce peripheral tolerance to myelin antigens [29] and then to be capable of protecting neural cells through paracrine mechanisms [50*] and even inducing local oligodendrocyte precursor proliferation [44**]. Thus, a common signature defines the therapeutic plasticity of adult stem cells based on shared bystander activities, namely immunomodulation, neuroprotection and induction of endogenous neurogenesis (Fig. 1).

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