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Using stem cells in multiple sclerosis therapies

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Stem cell transplantation approaches offer for the first time the opportunity to design therapeutic approaches for multiple sclerosis (MS) with curative intent. Here we discuss key observations and questions emerging from clinical trials of hematopoietic stem cell transplantation for MS and from studies of myelin/neural repair in experimental models of demyelinating disorders.

Introduction

Multiple sclerosis (MS) is an inflammatory, demyelinating disease with various degrees of axonal damage. MS leads to substantial disability through compromise of sensory, motor, autonomic and neurocognitive functions in young adults and affects women about twice as often as men. The disease etiology is not known, but a complex genetic trait with multiple, weakly associated genes and a number of putative infectious triggers are thought to contribute to disease expression. As a result, MS presents with different disease courses and clinical heterogeneity. From a pathogenetic perspective, the main component is thought to be a T-cell mediated autoimmune response that induces and perpetuates a series of other inflammatory/immune events. However, central nervous system (CNS)-specific factors, such as increased vulnerability to inflammatory tissue injury and reduced ability for repair, are equally important. The interplay between these factors initially results in a relapsing-remitting disease course (Figure 1), during which inflammatory bouts are either repaired or functionally compensated, but over time a secondary chronic progressive disease course evolves in most cases. During the latter phase, inflammation gradually declines, and features similar to degenerative neurologic diseases ensue. A smaller fraction of patients presents with primary progressive disease. For the purpose of this report, the distinction between initial inflammatory disease processes and parallel or subsequent degenerative events is critical. While immunomodulation and immunosuppression have shown some effects during relapsing–remitting (RR-MS) disease or the early secondary progressive (SP-MS) course, these therapies proved useless at stages when the degenerative aspects predominate or when too much CNS tissue has been damaged to allow for functional compensation. With the recent progress in stem cell and growth factor biology and with a better understanding of normal myelin formation, pathologic demyelination, remyelination and axonal damage, we have begun to consider therapies that aim at neuroprotection or even structural repair of CNS damage. Following previous experiences of inefficient immunomodulatory therapies in the neurodegenerative stage of MS, we anticipate that CNS repair therapies will be similarly frustrating unless we can inhibit the autoimmune disease process at the same time. Based on these considerations, we argue here that curative therapies in MS should aim at a combination of effective immunomodulation or reestablishment of tolerance, and either neuroprotection or transfer of neural/glial progenitor or stem cell populations. Stem cells may assume an important place in both stages of treatment.

The advent of immunomodulatory therapies has considerably increased the treatment options available for patients with MS in the last decade. Controlled clinical trials demonstrating the efficacy of IFN, glatiramer acetate and mitoxantrone on inflammatory disease activity have led to approval of these drugs for the treatment of MS by most academic associations and government agencies. Several newer immune-modifying agents are at various stages of evaluation. Nevertheless, immunomodulatory treatments are not tolerated by all patients, they are effective only in a proportion of patients, and the beneficial effects are limited to the duration of treatment. In addition,

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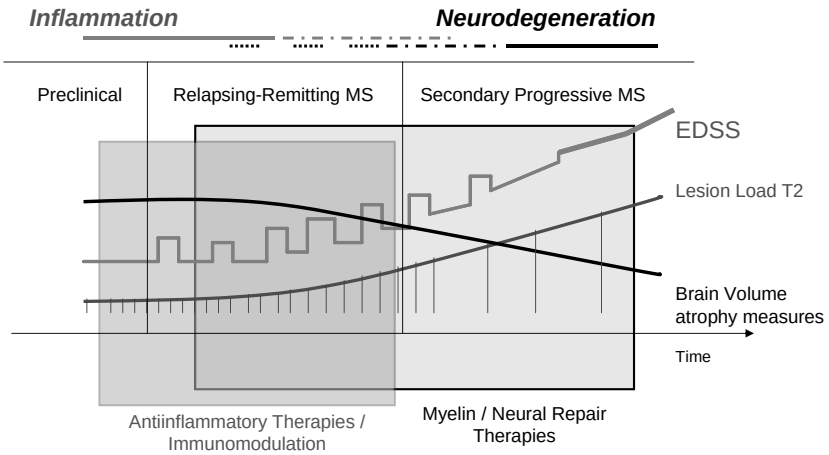

Figure 1. *Two overlapping pathogenetic components and therapeutic strategies in MS.*

the high cost of continuous treatment with proprietary drugs limits access to treatment of otherwise eligible patients in several countries. For all these reasons we believe that immunomodulatory therapies should be seen as an intermediate step towards the development of more radical, curative treatments. Hematopoietic stem cell transplantation (HSCT) is the most promising curative approach for MS currently being evaluated. The present report summarizes the key points emerging from clinical experiences, relevant to the design of new immune-conditioning and cell-based myelin repair therapies that could, alone or in combination, achieve prolonged or permanent remission and partial or complete neurologic recovery, providing at least a functional cure of MS.

HSCT to stop inflammatory lesion formation in MS

Clinical trials of autologous HSCT for MS have been reviewed recently in detail [1,2]. Two main lessons have emerged from these studies:

- high-dose immunoablative treatment can stop the formation of new lesions in the vast majority of patients with aggressive MS;
- progression of disability often continues in patients with advanced disability at the time of treatment.

A landmark multicentric clinical trial conducted by Mancardi *et al*. [3] provides the strongest evidence to date suggesting that myeloablative autologous HSCT can completely suppress magnetic resonance imaging (MRI) evidence of active disease in MS patients. This study employed triple-dose gadolinium-enhanced MRI to document active disease as an inclusion criterion for the trial, and to monitor the effects of treatment on inflammatory disease activity. Remarkably, no gadolinium-enhancing lesions were detectable in any of the 10 patients within 4 months of completion of HSCT and for a median published follow-up of 15 months. An update of this clinical trial, published in preliminary form, confirmed the absence of gadolinium-enhancing lesions in 17 patients with long-term follow-up post-therapy (median 41 months, range 8–65) [3]. Similar results showing an absence of enhancing lesions in 14 patients at 1-year post-therapy have been reported by a Spanish group [4,5]. These data prove that high-intensity (myeloablative) conditioning regimens can completely suppress local blood–brain barrier disruption, thought to reflect acute inflammation in MS. In contrast to these extremely encouraging findings, an Italian collaborative group showed that brain atrophy, based on MRI measurements of normalized brain volume changes, continued to progress in hematopoietic stem cell-transplanted MS patients with a decrease of 1.87%/year [6], a nearly identical rate to that reported by Nash *et al*. [7] for total brain volume (-1.84%). In the absence of a control arm, the investigators were unable to conclude whether HSCT may have exerted a beneficial, detrimental or no effect on the development of brain atrophy. These results are in line with those from two reports of clinical trials that showed the persistence of clinical deterioration in patients with progressive MS and advanced disability (expanded disability status score, EDSS [8], >6.0) who received HSCT [7,9]. These observations suggest that blocking

inflammation may not be sufficient to arrest progressive clinical worsening consequent to oligodendroglial or axonal degeneration, or both. Based on natural history studies and clinical trials of treatment agents, most believe that irreversible dystrophic and degenerative processes leading to axonal loss represent the main cause of disability in patients with secondary progressive MS, particularly in its later stages (Figure 1). Abnormalities in normal-appearing white matter detected by non-conventional MRI techniques have suggested that degenerative or at least non-acutely inflammatory pathogenic processes can coexist with inflammatory activity even in the earlier, relapsing–remitting phases of MS. The exact nature of these diffuse changes and its relationship with ongoing inflammatory activity, however, is poorly understood. A major unanswered question is also whether, and at which exact stage of disease, the eradication of inflammatory disease activity can prevent the initiation of progressive degenerative processes. Because of its powerful suppressive effects on acute inflammatory disease activity, HSCT is the best candidate therapy to address this question. As the clinical trials conducted until now have almost exclusively enrolled patients with secondary progressive MS and high disability (EDSS 6.0 or greater), available data do not allow drawing any conclusions on this issue. Interestingly, in the recent reports by Burt *et al.* [9] and Nash *et al.* [7], the only two patients (one from each trial) that improved their disability status after treatment (by 2.5 and 0.5 EDSS points, respectively) were the only patients with RR-MS.

Additional strategies for reinduction of tolerance in MS through acute immunosuppression are being evaluated or considered. These include high-dose immunosuppressive treatment without stem cell support [10], non-myeloablative HSCT [11] and reduced intensity conditioning followed by allogeneic HSCT [12]. Reviewing these therapeutic approaches is beyond the scope of this article. A comparative summary of their main features is presented in Table 1.

Taken together, the experience in clinical trials of autologous HSCT for the treatment of MS prompts us to generate the hypothesis that complete abrogation of inflammatory disease activity achieved by high-dose immunoablation and HSCT may lead to long-term clinical stabilization and allow partial functional recovery when applied early in the course of disease and prior to significant accumulation of disability (EDSS 4.0) in patients with RR-MS. As discussed later, this stage of disease offers the best prospects for curative approaches combining the eradication of inflammatory disease activity with therapeutic strategies aimed to promote neural/glial repair in the CNS.

Remyelination and axonal repair strategies

The concept of using stem cells, which are clonal, self-renewing, pluripotent, progenitor cells, to regain function

Table 1. High-dose immunoablation/HSCT strategies to stop inflammatory disease activity and restore immune tolerance in MS

Treatment	Therapeutic advantage	Risks or difficulties
High-dose immunoablative (myeloablative) autologous HSCT	Reasonable risk–benefit ratio only in patients with aggressive MS refractory to other treatments	Autologous stem cells or residual lymphocytes in hematopoietic graft may carry risk of reconstituting disease-mediating immune cells. Optimal intensity of regimen unknown
Reduced intensity non-myeloablative autologous HSCT	Safer than high-intensity regimen, could be applied to patients at earlier stages and with milder forms of MS	Trials are in early stages and efficacy is unknown. Elimination of disease-mediating immune cells might be less complete compared with high-dose regimens
Allogeneic HSCT	Complete donor chimerism of immune cells may reduce genetic susceptibility to MS. Mixed chimerism may exploit graft-vs.-autoimmunity effect for tolerance reinduction	Chronic GvHD may significantly affect clinical outcome and require long-term immunosuppressive treatment
High-dose immunosuppressive treatment without stem cell transplantation	Practical advantages of simpler procedure not requiring stem cell collection, processing and administration	Safety and long-term efficacy in MS remains to be determined. May not achieve eradication of disease-mediating immune cells

of damaged areas of the brain is attractive and so far supported by several studies. In the CNS of MS patients, the recurrent immune attacks lead to multi-focal damage to oligodendrocytes and neurons and pose particular challenges to optimizing an effective repair intervention. Any intervention must be aimed both at stopping the pathologic immune reaction that results in the formation of a lesion, as discussed in the previous paragraphs, as well as readily replenishing the lesion with stem cells that can reverse the loss of function. Although immune-mediated demyelination is thought to be the primary event, axonal damage is also an early pathologic finding in normal-appearing white matter and early inflammatory lesions [13]. The cell bodies of neurons and oligodendrocytes appear to be relatively protected against overt inflammatory damage. However, their neurites and the myelinating processes are very sensitive to toxic inflammatory mediators because of their small size and high metabolic activity. The spontaneous remyelination that occurs as a physiologic response in the early phases of disease has a major function in protecting axons from further damage [14,15]. This response, however, tends to fail at later stages, partially as a consequence of glial scarring and extensive microglial activation. While numbers of mature oligodendrocytes and progenitor cells progressively decline, Wallerian degeneration of axons becomes the major contributor to the progression of disability over time [16]. Clearly, remyelination is the process that has to be enhanced in order to preserve the neuronal function at later stages as neuronal-replacement therapies are unlikely to have an impact due to the inhibition of axonal sprouting intrinsic to the mature nervous system [17]. Prolonging the duration of endogenous reparative processes pharmacologically has been shown to be promising in animals [18].

Advances in the study of several animal models of genetic, chemically induced or immune-mediated CNS demyelination prove that functional remyelination can be achieved by transplanting exogenous cells into the lesioned nervous system. A variety of pluripotent cell types that have potential clinical applications have so far been identified (Table 2). Most of them have proven to be safe and somewhat effective in repairing the damaged myelin tissue, leading to an improvement of the underlying clinical conditions (e.g. restore axonal conduction) [19]. Notably, other than through the direct differentiation into the cell type of need, transplanted stem cells may exert positive effects on oligodendrocyte progenitors and neurons through local production of survival and growth factors [20]. As soon as reliable protocols for optimal cell-expansion can be developed for human cells, supplementation of large numbers of autologous cells capable of providing a robust trophic support might become therapeutic.

Interestingly, many of the receptors involved in homing of activated lymphocytes and monocytes to the brain in an inflammatory attack are also constitutively expressed on neural stem cells [21]. Once injected into the blood stream, these cells have the ability to reach damaged areas of the blood–brain barrier [22], most probably in response to

Table 2. Cell-based therapy approaches to promote myelin and axonal repair in MS

Cell type	Source/origin	Differentiation/therapeutic potential
Embryonic stem cells	Isolated from the inner cell mass of the developing embryo	Able to differentiate into all embryonic and adult cell types. Using somatic nuclear transfer strategies, immunocompatible cells can potentially be obtained
Neural precursor cells	Multipotent cells that can be isolated from several regions of the developing embryonic brain	Can be induced to differentiate *in vitro* into oligodendrocytes, astrocytes and neurons. Upon engraftment into the irradiated animal, these cells have also the capability of giving rise to all blood lineage cells *in vivo*
Adult neural stem cells	A relatively small population of cells present in the adult white and gray matter	These cells have significant replicative potential and are thought to be capable of differentiating into any neural cell
Adult BM stem cells	BM is an accessible source of autologous hematopoietic and mesenchymal stem cells	These cells show extensive differentiation potential into cells of many other organs, including the brain

the same gradients of chemokines and cytokines that exert influences on the immune system. The intravenous route of cell administration is attractive because it is probably safer and more effective than any other procedure designed to reach simultaneously all the inflamed areas of the brain. It should be stressed, however, that for the same reasons discussed above cell-based therapies alone are not likely to achieve any significant improvement of silent/chronic lesions.

Because of its silencing effect on the immune system, hematopoietic (together with neural) stem cells transplantation could provide the most effective treatment if performed at early stages of multiple sclerosis development.

Conclusions and future directions

The above considerations provide only a rough framework regarding which directions need to be examined toward an effective therapy of MS. Clearly, we will not be able to identify a treatment that fits all MS patients equally. Efficacy will depend on the stage of the disease, the extent and type of inflammation, the amount of CNS tissue damage, and other factors. Ideally, one will aim at early and effective therapies that completely shut down the autoimmune inflammatory process in patients who are still fully functional and have little CNS damage. HSCT currently appears the best option; however, improvements of the conditioning regimen, identification of the best patient population and disease stage for this procedure, as well as the question of autologous vs. allogeneic HSCT, need to be examined. Subsequent to HSCT, effective neuroprotection might become an important adjunct. In patients with more advanced disease and disability because of brain or spinal cord damage, a combination of HSCT with local or systemic delivery of growth factors, stem/progenitor cells and, for example, approaches that neutralize inhibitory signals in the CNS such as Nogo [23] will be preferable, but we need to learn much more about these factors, i.e. which growth factor, at which times and in conjunction with which cell type.

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