

Immunological questions on hematopoietic stem cell transplantation for multiple sclerosis

PA Muraro and R Martin

Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

Summary:

Multiple sclerosis (MS) is considered an inflammatory autoimmune disorder. Approved immunotherapies are only moderately effective in reducing disease exacerbations and brain inflammation in a subset of patients. Autologous hematopoietic stem cell transplantation (HSCT) has emerged in recent years as the first opportunity to offer to patients a radical, potentially curative treatment. Here, we will summarize key immunopathological aspects of MS and discuss important questions that need to be addressed to clarify the therapeutic role and mechanism of action of HSCT in this disorder.

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MS: an inflammatory demyelinating and degenerative axonal disorder

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disorder of the central nervous system (CNS) that predominantly affects young adults. Approximately one million individuals worldwide are affected, over 200 000 only in the US.¹ Females are affected twice as frequently compared to males. Impairment of motor function with paralysis and spasticity, incoordination (ataxia), loss of vision, sensitive disturbances, paroxysmal pain, sphincter dysfunction and cognitive impairment are the most common symptoms. The clinical course is very heterogeneous, the most typical being a relapsing-remitting form with exacerbations followed by complete or partial recovery of neurological function. More advanced stages of the disease are characterized by accumulation of

disability, either from incomplete recovery from relapses or by continuous progression (secondary progressive MS). Less commonly, the disease presents from the beginning with a slowly progressive course (primary progressive MS), with or without relapses.²

MS pathology selectively affects the CNS. Focal lesions can be found in the white matter of the brain, of the brainstem, of the cerebellum and of the spinal cord. The pathological hallmark of MS is the demyelinated plaque, characterized by varying grades of myelin loss and gliosis. Prominent intraparenchymal and perivascular inflammatory cell infiltrates, predominantly lymphocytes and macrophages, are commonly found in active lesions. Axonal damage has been recognized as an additional important component of MS pathology.³ Different patterns of demyelination have recently been associated to distinct possible pathways of immune-mediated tissue destruction.⁴ The interindividual heterogeneity of MS lesions suggests that different mechanisms may act in different patients, accounting for the variability of clinical courses, of immunological findings and of responses to treatments.

The cause of MS is unknown and the pathogenic process is incompletely understood. Several features of the disease, which include the presence of immune cells in lesions, cerebrospinal fluid (CSF) abnormalities with intrathecal production of oligoclonal IgG, the response to immunomodifying treatments and the increased disease susceptibility of individuals with certain HLA class II haplotypes, point to an immune-mediated pathogenesis. Current knowledge supports a T-cell-dependent, T- and/or B-cell-mediated autoimmune pathogenesis targeting myelin components or myelin-producing cells.⁵ Myelin proteins that could be involved as targets of the inflammatory immune response in MS include myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendroglia glycoprotein (MOG) and 2',3'-cyclic nucleotide 3' phosphodiesterase (CNPase) as well as other minor components. This hypothesis is supported by observations in experimental animals models. Immunization of susceptible animal strains with myelin antigens or transfer of myelin antigen-reactive T cells induces experimental autoimmune encephalomyelitis (EAE), an inflammatory disorder of the CNS which resembles MS. EAE studies have demonstrated that inflammatory demyelinating CNS disorders can be mediated by CD4⁺ myelin-specific T cells. In this context, disease-inducing T cells are clearly biased toward

Correspondence: Dr. PA Muraro, Neuroimmunology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bldg. 10, Room 5B16, 10 Center Dr MSC1400 Bethesda MD 20892-1400 USA

a proinflammatory T helper type 1 (Th1) phenotype. Likewise, in MS myelin-reactive Th1 CD4⁺ T cells may become activated in secondary lymphoid organs upon cross-reactive stimuli (molecular mimicry hypothesis), migrate through the blood–brain barrier (BBB), recognize myelin antigens and trigger inflammation. However, the highly dynamic nature of antimyelin T-cell reactivity demonstrated in several studies^{6–8} as well as the difficulty in safely obtaining tolerization via Altered Peptide Ligand (APL)-based treatment⁹ have raised questions about the usefulness of antigen-specific immunotherapy for MS.

Magnetic resonance imaging (MRI) findings in the initial stages of MS lesion development suggest an important role of BBB dysfunction in the inflammatory changes of the CNS.¹⁰ Blood Brain Barrier (BBB) dysfunction alone, however, is not sufficient to start an inflammatory response toward myelin constituents. In fact, resident CNS cells in normal conditions lack major histocompatibility (MHC) molecules, thus preventing the initiation of antigen-specific immune responses. This state of immunological ignorance can be altered by the induction of MHC expression,¹¹ which can occur during inflammation. Following the activation of autoreactive T cells in the CNS, secretion of proinflammatory cytokines and chemokines may recruit additional T cells and B cells into the lesion. Different mechanisms may be involved in the effector stage of demyelination, such as cytotoxicity and the action of cytokines and tumor necrosis factors. Activated macrophages can contribute to myelin destruction through several mediators including cytokines, reactive oxygen intermediates, proteases and lipases. Myelin-specific autoantibodies may provoke tissue damage by antibody-dependent cell-mediated cytotoxicity and by the activation of complement.¹² Axonal damage has been recognized as an additional important component of MS pathology.³ In fact, neuronal degeneration is likely to play a major role in determining disability, with an increasingly relevant weight as disease progresses towards secondary chronic stages.

Exploring the immunological mechanisms of HSCT in MS

No approved treatments currently offer curative options for patients with MS.¹³ While mortality risks of HSCT for the treatment of MS cannot be understated,^{14,15} clinical results of Phase I/II trials^{15–18} seem overall encouraging, particularly when objective MRI measures have been employed to monitor the evolution of inflammatory disease activity.^{19,20} These results have prompted the design of controlled trials of HSCT *vs* standard therapy (mitoxantrone), which are planned to start in Europe and in the US in the near future. These studies are required to obtain a denominator in the risk/benefit equation before any assumption can be made on the future role of HSCT for MS.

While clinical experience has been rapidly accumulating in the last few years, little is known on which changes in the immune system induced by HSCT are responsible for favorably affecting the course of MS. Three nonmutually exclusive hypotheses can be formulated, namely: (1) immune ablation can eliminate a pathogenic immune response; (2) HSCT can reconstitute an immune system with a new or restored immune tolerance; and (3) differentiation of hematopoietic stem cells into glial and neural precursors can contribute to restoring damaged nervous structures. Table 1 presents a few important questions that are related to these hypotheses and the status of current knowledge. As evident from the table itself, available knowledge on these issues is quite limited. In fact, little data have been reported on immune function after HSCT in MS. Fassas *et al*²¹ treated 15 patients with progressive MS (eight primary progressive, and seven secondary progressive) with autologous HSCT. A profound fall of CD4⁺ cell counts and an increase in CD8⁺ cells were observed in all patients. Burt *et al*¹⁶ reported on six patients with progressive MS treated with HSCT. Markedly reduced CD4⁺ cell counts were observed during the first 12 months post transplant, with inverted CD4/CD8 ratios. CD4⁺ cells had almost exclusively a CD45RA⁻

Table 1 Key hypothesis and questions on immunological mechanisms of HSCT for MS

Hypothesis	Questions	Current status
Immune ablation eradicates the pathogenic immune response in MS	Are autoreactive cells deleted or their frequency reduced after transplant?	Unclear. Possibly reduced ¹⁴
	Are there phenotypic or functional changes, Th1 to Th2 cytokine phenotype shift, downregulation of adhesion molecules, chemokine receptors, costimulatory molecules) of self-reactive cells after HSCT?	Unknown
HSCT can generate a newly tolerant immune system	Is regulatory T-cell function (CD8 ⁺ CTL, CD4 ⁺ CD25 ⁺ T cells) improved by HSCT?	Unknown
	Are there global changes in the cytokine environment that promote tolerance (ie generalized shift of Th1 to Th2 responses)?	
	Are there global changes in other relevant aspects of immune homeostasis, such as costimulatory and apoptotic pathways?	
Stem cell transplant can promote repair of CNS lesions	Pluripotent stem cells from the graft can differentiate into glial/neural precursors <i>in vivo</i>	Possible from extensive data in animal models and from recent data in humans ^{22–24}
	Stem cells can be manipulated <i>in vitro</i> to boost their regeneration-promoting potential	Controversial

'memory' phenotype early post transplant. T-cell proliferative responses to polyclonal stimulation were reduced in the post transplant period. Openshaw et al¹⁴ transplanted and conducted immunological studies in five patients with secondary progressive MS. Lymphocyte proliferation assays evaluating responses to a panel of 14 MBP peptides and a set of five immunodominant myelin protein peptides (MBP 84–106; MBP 142–163; PLP 104–117; PLP 142–153; MOG 42–53) were performed in three patients. Some responses to myelin peptides were suppressed after HSCT. Data from a multicenter analysis recently confirmed the previous observations of reduced CD4⁺ cell counts.¹⁵ These data altogether provide preliminary evidence of suppression of autoreactive cells during the lymphopenic (particularly CD4 depleted) post-transplant phase. Even less information is available on how B-cell and plasma cell responses may be affected by HSCT. Limited available data suggest that intrathecal synthesis of oligoclonal IgG, a characteristic feature of MS, is not eliminated by HSCT. In one report, CSF obtained from two patients at 1 year after transplant showed persistence of oligoclonal bands.¹⁴ In another study, the same oligoclonal band pattern of one MS patient's CSF was shown to persist post-transplant.²⁰

In summary, there are currently no data offering sufficient insight on how immune tolerance may be reconstituted in MS patients after transplant. To gain further knowledge on this matter, we are currently conducting immunological studies on patients with MS who receive HSCT, directing our efforts to address the main questions outlined in Table 1. Combining careful clinical/paraclinical follow-up studies of MS patients treated with HSCT and detailed immunological investigations may, in fact, offer a unique opportunity to improve our understanding both of HSCT and of the pathogenesis of the disease. For example, the identification of different pre- and post-transplant myelin-specific T cell repertoires (or different specific phenotypes) in patients who received effective HSCT may help to identify disease-mediating cell populations. In contrast, if the same myelin-reactive T-cell repertoire (including its functional phenotype) were reconstituted in patients for whom HSCT resulted in successful clinico-pathological remission, its importance in the pathogenic process would become questionable. If, on the other hand, disease progression or relapses resume after transplant in some patients, characterization of the thymic vs peripheral origin of autoreactive T cells may answer the critical question, whether persistence or reoccurrence of disease was due to spontaneous reconstitution or to incomplete immunoablation of pathogenic T cells. In the latter case, transplant procedures that allow a complete eradication of pathogenic T cells should be preferred.

Conclusions

Once the efficacy of HSCT has been confirmed in a controlled setting, the procedure could be envisioned as a curative option to be considered early during the disease course for patients with a strong inflammatory component. Combined immunological studies are expected to yield valuable information on important aspects of therapeutic

mechanisms of transplant. In more advanced stages of disease when disability has accumulated, HSCT may still represent the most powerful therapy to achieve complete immunopathological disease remission. This seems a logical prerequisite condition before attempting stem cell-mediated neural repair, a captivating idea that has recently become conceivable.^{22–24}

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