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Hematopoietic Stem Cell Transplantation for Multiple Sclerosis

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Hematopoietic stem cell transplantation (HSCT) was proposed as a treatment for multiple sclerosis (MS) in 1995 based on favorable results in animal models including experimental autoimmune encephalomyelitis.[1] These initial or first-generation trials were developed by medical oncology subspecialists, used malignancy-specific myeloablative transplantation regimens, and selected patients with secondary progressive MS with rapid progression of disability. In general, these trials suffered from higher than anticipated toxic reactions including treatment-related and disease-related mortality, continued loss of brain volume as seen on magnetic resonance imaging (MRI), and, at least in some patients, continued progressive disability despite marked attenuation or absence of gadolinium-enhancing lesions on MRI. Learning from these experiences, second-generation transplantation trials for MS are using MS-specific nonmyeloablative transplantation regimens and selecting for active relapses despite the use of interferon treatment in patients with less accumulated disability. While still preliminary, results using second-generation nonmyeloablative HSCT regimens are encouraging with minimal treatment-related morbidity and improvement in Expanded Disability Status Scale (EDSS) scores. The following 3 variables seem important in predicting the benefit and minimizing the toxic effects from an autologous stem cell transplantation in patients with MS: the selection of patients who still have inflammatory disease (ie, gadolinium enhancement on MRI and/or frequent active relapses), treatment early in the course before the onset of significant irreversibly progressive disability, and the use of a safer lymphoablative but nonmyeloablative HSCT conditioning regimen.

RATIONALE OF HSCT

The current therapies for MS consist of immune-modulating agents, such as interferons or glatiramer acetate, and anti-inflammatory and immune suppressive drugs such as glucocorticoids, methotrexate, and mitoxantrone (Novantrone; Immunex Corporation, Seattle, Wash).[2] Autologous HSCT (either myeloablative HSCT or nonmyeloablative HSCT) is a form of immune suppressive therapy in that immune suppression is maximized to the point of transient immune ablation. In theory, the transplantation conditioning regimen ablates the aberrant disease causing immune cells while hematolymphopoietic stem cells (HSCs) regenerate a new and antigen naive immune system. Therefore, all of the toxic reactions and efficacy of an autologous HSCT (either myeloablative HSCT or nonmyeloablative HSCT) is likely a consequence of the conditioning regimen.

ANIMAL RESULTS

Experimental autoimmune encephalomyelitis is an autoimmune demyelinating disease of the central nervous system (CNS) induced by either in vivo immunization with myelin peptides or by adoptive trans-

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fer of ex vivo primed $CD4^+$ T cells. Hematopoietic stem cells are acquired from a euthanized animal of a different animal strain (allogeneic HSCT), from one of the same highly inbred strain (syngeneic HSCT), or from a syngeneic animal with the same stage of disease (pseudoautologous HSCT). Any 3 donor HSC sources (allogeneic, syngeneic, or pseudoautologous) are capable of inducing remission and preventing relapse when performed during the acute phase of MS.[3-8] In contrast, HSCT is ineffective therapy for late-stage or chronic progressive experimental autoimmune encephalomyelitis.[3]

Theiler murine encephalomyelitis virus (TMEV) induces a CNS demyelinating disease manifest at onset as progressive neurologic deterioration. Theiler murine encephalomyelitis virus is a small RNA virus (picornavirus) acquired in the wild by oral inoculation. Disease-resistant strains of mice clear the virus within 2 weeks of infection, while disease-susceptible strains have a persistent CNS infection. Both virus- and myelin-specific T-cell responses occur in TMEV-induced demyelinating disease.[9] Unlike the beneficial effect of HSCT seen in relapsing experimental autoimmune encephalomyelitis, syngeneic HSCT of TMEV-infected mice results in exacerbation of neurologic disability and high mortality due to CNS viral hyperinfection following immune ablation.[10] Therefore, a functional immune system appears important to prevent lethal neuropathic effects from a persistent viral-induced CNS demyelinating disease. Since several hundred patients with MS have undergone HSCT worldwide without experiencing viral encephalomyelitis, it is unlikely that patients with MS harbor a persistent neuropathic viral infection.

In summary, animal models such as experimental autoimmune encephalomyelitis and TMEV-induced demyelinating disease suggest that (1) MS is an autoimmune-initiated disease similar to experimental autoimmune encephalomyelitis and not a persistent viral-related demyelinating disease akin to TMEV and (2) to be effective, HSCT should be performed in the relapsing phase of MS while it is still an immune-mediated inflammatory process rather than in its chronic progressive phase when axonal degeneration predominates.

MOBILIZATION OF HSCs FROM PATIENTS WITH MS

The most common method of collecting HSCs is by mobilization from the peripheral blood. Since negligible HSCs are detectable in the peripheral blood during the steady state, either a hematopoietic growth factor such as granulocyte colony-stimulating factor or chemotherapy (usually cyclophosphamide) with or without granulocyte colony-stimulating factor is necessary to mobilize HSCs into and subsequently collect HSCs from the blood. Hematopoietic growth factors used to mobilize HSCs also have immune-modulating effects and unlike malignancies may exacerbate disease depending on the growth factor. Granulocyte colony-stimulating factor may precipitate clinical flares of MS sometimes with significant and irreversible neurologic deterioration.[11,12] Colony-stimulating factor–induced MS flare may be prevented by either administration of corticosteroids or mobilization using the combined therapy of cyclophosphamide and granulocyte colony-stimulating factor.

EX VIVO HCS SELECTION

Most mononuclear cells collected by peripheral blood apheresis are immune cells such as lymphocytes and monocytes not HSCs. While the true identity of human HSCs remains elusive, either purified $CD34^+$ or $AC133^+$ hematolymphopoietic progenitor cells are sufficient for hematopoietic and immune reconstitution. In general, a minimum number of 2×10^6 $CD34^+$ cells per kilogram of recipient weight will ensure engraftment. Hematopoietic stem cells may be positively selected or enriched ex vivo using antibodies to $CD34^+$ or AC133 or purified by negative selection by using antibodies to remove lymphocytes. In practice, the most common method of purging lymphocytes is via CD34-positive selection using either the Miltenyi CliniMACS (Bergish Gladbach, Germany) or the Baxter Isolex (Deerfield, Ill) cell separator device. Whether enriching the graft for $CD34^+$ HSC is necessary or even superior to infusion of an unmanipulated graft remains unclear. $CD34^+$ selection by removing lymphocytes is perhaps best viewed as another method of immune suppression. For an intense conditioning regimen, $CD34^+$ selection may be unnecessary or even detrimental by increasing the risk of treatment-related infection.

CONDITIONING REGIMEN

The rationale for autologous HSCT of MS is to regenerate an antigen-naive immune system from the patient's own HSCs. Therefore, the goal of the conditioning regimen is lymphoablation not myeloablation. The autologous HSCT regimen should be based on immune suppressive drugs that are well tolerated at conventional nontransplantation doses and are expected to remain safe and nonmyeloablative at higher transplantation doses. The regimen must also avoid further damage to already injured axons and oligodendrocytes. By definition, myeloablative agents are lethal to HSCs and, apart from their myeloablative effect on bone marrow, may be similarly cidal to tissue-specific stem cells such as oligodendrocyte progenitor cells or neural stem cells. In animal models, cranial irradiation impairs the mechanism of CNS repair by neural stem cell apoptosis, alteration in cell cycle progression, and/or destruction of the neural stem cell niche or milieu through invasion of macrophages and microglia.[13] This raises concerns about using total body irradiation based, or any other stem cell ablative regimen, in the treatment of MS.

Nonmyeloablative HSCT regimens that are as immune suppressive as myeloablative regimens but without myeloablative adverse effects may be designed by using agents or combinations of agents such as fludarabine, cyclophosphamide, antilymphocyte antibodies such as CAMPATH-1H or antithymocyte globulin, and/or by the use of $CD34^+$ selection of the graft. Fever-related deterioration of neural function in MS, termed "pseudoexacerbations," due to conduction blocks in marginally functioning demyelinated axons should be avoided during

transplantation by minimizing pyrogenic agents in the conditioning regimen. Similarly, the risk of infection-related fever should be minimized during transplantation by use of prophylactic antibiotics.

In summary, for MS the rationale behind the HSCT conditioning regimen should be to (1) dose-escalate agents that work as conventional therapy, (2) maximize immune suppression without myeloablation, (3) avoid conditioning regimen agents that may cause injury to already disease-affected and damaged CNS tissue, (4) avoid injury to tissue-specific stem cell compartments that may be important for CNS repair, (5) minimize the risk of fever, and (6) design regimens that are justified for the risk of the disease being treated.

RESULTS OF FIRST-GENERATION HSCT PROTOCOLS FOR MS

Initial HSCT protocols generally did not follow the above concepts but rather used aggressive malignancy-specific myeloablative regimens in patients with progressive MS. From these studies, immune suppression following autologous HSCT appears to be an effective therapy to halt MRI lesion activity. In fact, there is no other therapy that may provide such a striking and long-term effect on suppressing MRI-enhancing activity and new T2-weighted lesions. Saiz et al[14] using a regimen of carmustine (BCNU), cyclophosphamide, antithymocyte globulin, and $CD34^+$ selection of the graft reported no post-HSCT–enhancing lesions and a decrease in mean T2-weighted lesion load by 11.8%. Mancardi et al[15] using a regimen of BCNU, etoposide, cytosine arabinoside, and melphalan (BEAM) performed triple-dose gadolinium-enhanced MRIs monthly for 3 months before HSCT and monthly for 6 months and then every 3 months after HSCT. Complete and durably suppressed MRI activity was documented following HSCT.

Autologous HSCT also appears to effectively reset the immune system. The mechanism of autologous HSCT–induced remission of an immune-mediated disease may be transient immune suppression–related lymphopenia, a more durable "immune reset" because of regeneration of an antigen-naive immune system from the HSCs, or both. By analyzing T-cell receptor repertoires with flow cytometry, polymerase chain reaction spectratyping, and sequenced-based clonotyping as well as new thymic T-cell emigrants by T-cell receptor excision circle, we have shown in patients with MS undergoing HSCT that a new and antigen-naive T-cell repertoire arises from the HSC compartment via thymic regeneration.[16] This suggests that intense immune suppression via HSCT results in long-term immune reset independent of persistence of immune suppression–mediated lymphopenia.

Despite suppression of MRI activity and encouraging immune-reconstitution data, the clinical outcome in terms of progressive neurologic disability is not obviously better than the natural history of patients with progressive MS. The discordance between promising immune analysis and MRI data vs continuing clinical disability is most likely due to selection for transplantation of patients with late progressive disease without ongoing CNS inflammation. In a European retrospective analysis of 85 patients, the progression-free survival at 3 years was 78% in secondary progressive MS and 66% in primary progressive MS.[17] At Northwestern University, Chicago, Ill, of 21 patients with secondary progressive MS treated using a myeloablative HSCT regimen, disease progression in more disabled patients with a pretreatment EDSS score of 6.0 or higher was significantly worse compared with those with an EDSS score below 6.0.[18] In fact, none of the 9 patients with an EDSS score below 6.0 had disease progression worsening by 1.0 or more EDSS points after more than 2 years of follow-up. The single patient in this study with relapsing-remitting MS not only failed to progress but also had a sustained improvement by 2.0 EDSS steps. In a Rotterdam, the Netherlands, study using a total body irradiation–based myeloablative regimen in patients with secondary progressive MS, 9 of 14 patients had continued posttransplantation progression of disability by EDSS rating (R.H, oral presentation at Multiple Sclerosis International Stem Cell Transplant [MIST] Trial meeting, April 2, 2005).

In retrospect, since autologous HSCT is a form of intense immune suppression, it is unlikely to beneficially affect the noninflammatory, that is, degenerative aspects, of MS. This is supported by MRI data in patients with progressive disease undergoing HSCT who have a continued decrease in brain volume suggesting continued axonal atrophy for the duration of reported follow-up, at least 2 years, after HSCT.[19] The importance of selecting patients with inflammatory disease is also supported in 2 patients with pretreatment malignant MS manifest by striking gadolinium-enhancing lesions and severe deficits (nonambulatory with EDSS scores of 7.5 and 8.0) after a short clinical duration of disease (1 and 3 years) who were able to ambulate 100 and 300 m, respectively, with only unilateral assistance by 6 months after HSCT using a BEAM conditioning regimen.[20]

Besides a continued decline in EDSS scores in patients with late progressive disease with some disease-related deaths,[17,18,21] the initial myeloablative HSCT regimens have been associated with treatment-related deaths.[17,22,23] A phase 1 study performed at the City of Hope, Duarte, Calif, using a maximum-dose myeloablative cancer regimen of busulfan and cyclopospham ide also known as "Big BuCy," along with antithymocyte globulin and $CD34^+$ selection resulted in treatment-related death in 2 of 5 patients.[22] A similar Big BuCy regimen performed in Ottawa, Ontario, resulted in a treatment-related death due to hepatic veno-occlusive disease in 1 of 11 patients (Mark Freedman, MSc, MD, FRCPC, oral communication, April 2, 2005). A slightly less intense regimen using a leukemia-specific protocol of myeloablative total body irradiation, cyclophosphamide, antithymocyte globulin, and $CD34^+$ selection performed at the Fred Hutchinson Cancer Center, Seattle, Wash, resulted in 1 reported transplantation-related death in 24 patients.[23] A similar irradiation-based regimen performed in Rotterdam that enrolled 14 patients ended with 1 patient developing irradiation-related preleukemic myelodysplasia (R.H., presentation at MIST Trial meeting, April 2, 2005). In a retrospective European analysis of 85 patients treated with a lymphoma-specific regimen (BEAM, the least intensive of the cancer-specific myeloa-

blative regimens), 5 treatment-related deaths were reported.[17] The BEAM regimen which was also used in the Brazilian MS transplantation trial was recently replaced by cyclophosphamide and antithymocyte globulin owing to excessive BEAM-related morbidity and mortality (J.C.V., oral presentation at MIST Trial meeting, April 2, 2005). While an Italian trial of the BEAM regimen in MS showed better safety with no deaths in 19 patients,[24] the overall high transplantation-related mortality, mostly due to infection but also end-organ damage and treatment-related leukemia, has resulted in termination of trials, dose reduction in conditioning regimen drug intensity, enrolling less disabled patients, and/or limiting the procedure to more experienced centers. Nevertheless, significant concerns remain as to whether any of these first-generation myeloablative cancer-specific regimens are capable of achieving equipoise in a disease of low mortality such as MS, especially because the patients who are likely to benefit are generally not severely disabled.

SECOND-GENERATION NONMYELOABLATIVE HSCT PROTOCOLS FOR MS

The rationale for autologous HSCT for an immune-mediated disease is that the disease is not a genetic stem cell defect but rather a disorder triggered by an environmental component. For MS the logical goal of an autologous HSCTconditioning regimen is, therefore, immune ablation not myeloablation. Following nonmyeloablative HSCT, autologous HSCs are infused to shorten the duration of conditioning regimen–related cytopenias. Compared with myeloablative regimens, nonmyeloablative HSCT regimens have a lower treatment-related mortality, which in terms of the risk benefit from treatment is a significant advantage for MS that has a significantly lower disease-related mortality than malignancies. Despite the lack of myeloablation, too aggressive a combination of nonmyeloablative HSCT agents are highly immune suppressive that could also result in lethal opportunistic infections. Therefore, nonmyeloablative HSCT regimens must be tailored for the degree of immune suppression desired. Agents like etoposide, total body irradiation, busulfan, melphalan, or carmustine treatments that are used in myeloablative first-generation HSCT studies have nothing to do with treating MS but were chosen because of their familiarity by oncologists in treating cancer. In comparison, nonmyeloablative HSCT regimens use lymphoablative agents like cyclophosphamide and CAMPATH that neurologists already use to treat MS and that have little nonlymphopoietic toxic effects.

The goal of nonmyeloablative HSCT therapy is to prevent inflammation and suppress relapses by intervening before onset of irreversible progressive axonal degeneration. Rather than selecting for rapidly progressive disease, that is, an increase in the EDSS score of 1.0 or more points in the preceding 12 months, as performed in prior myeloablative studies, candidates for nonmyeloablative HSCT are selected for active inflammation. Criteria may include relapsing-remitting or relapsing-progressive MS with multiple acute relapses despite interferon treatment and gadolinium-enhancing MRI lesions with less accumulated disability (EDSS score, 2 5-6.0). Patients with higher EDSS scores could be considered if they have malignant MS manifest by rapid clinical deterioration and striking gadolinium enhancement. In Berlin, Germany, a nonmyeloablative HSCT protocol of cyclophosphamide and rabbit antithymocyte globulin has been used in children with marked improvements in EDSS scores, again with little morbidity (J.H. and R.A., oral presentation at MIST Trial meeting, April 2, 2005). At Northwestern University a regimen of cyclophosphamide and CAMPATH has been well tolerated with little morbidity and no infections in adults again with subsequent improvement in the EDSS score in most patients. In fact, 50% of the patients do not even require a red blood cell transfusion (R.K.B., oral presentation at MIST Trial meeting, April 2, 2005). Immune reconstitution and mechanistic studies of patients with MS treated with this regimen are underway and are expected to elucidate its mode of action also through a comparison with data on myeloablative HSCT.

SUMMARY

Hematopoietic stem cell transplantation is a form of immune suppressive therapy. Since the art of immune suppression is finding balance, oncology-trained transplantation physicians may need to reconsider the rationale for applying malignancy-specific regimens and procedures to MS.[25] On the other hand, HSC therapy to halt immune-mediated demyelination is neither theoretical nor if performed with anonmyeloablative HSCT regimen, fraught with the same morbidity or mortality as myeloablative HSCT. Nonmyeloablative stem cell transplantation stands on sound theoretical, scientific, and empirical foundations as meaningful therapy for refractory and breakthrough MS with ominous prognosis, still showing active inflammatory demyelination and a relative absence of axonal degeneration as the cause of disabilities. While the long-term durability of nonmyeloablative HSCT–induced remission of active inflammation is yet to be determined, it holds promise for patients with active inflammatory disease if performed before onset of significant irreversible axonal injury. The exact role of nonmyeloablative HSCT in the treatment of MS is being explored in a multicenter, multinational trial—the Multiple Sclerosis International Stem Cell Transplant (MIST) Trial.

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