High Dose Immunosuppression with Hemopoietic Stem Cell Support in the Treatment of Multiple Sclerosis

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High dose myeloablative chemotherapy with either allogeneic or autologous hemopoietic stem cell transplantation (support) is a standard step in the treatment of various hematological malignancies. It is also being explored in the management of certain solid tumors and autoimmune diseases [1]. This approach has two consequences. First, it produces severe suppression of hemopoiesis in bone marrow (myeloablation), which is subsequently restored by transplanted hemopoietic stem cells. Second, it leads to profound suppression of lymphopoiesis – immunoablation.

It has been known for a long time that immunoablation is crucial for conditioning before bone marrow transplantation for severe aplastic anemia, where autoimmunity plays a substantial role in the pathogenesis [2]. The myeloablative effect of high dose conditioning prior to allogeneic or autologous transplantation is used in the management of malignancies. On the other hand, the immunoablative effect, which can result in long-lasting immunosuppression, is used in the treatment of certain autoimmune diseases [3]. In the last few years, also non-myeloablative, primarily immunosuppressive conditioning regimens were succesfuly introduced in allogeneic bone marrow transplantation [4].

Bone marrow and peripheral blood progenitor cell transplantation

To restore hemopoiesis and immune system functions after high dose chemo/radiotherapy, hemopoietic cells obtained from HLA-matched individuals or autologous cells are used. Stem (progenitor) cells harvested from peripheral blood after mobilization are now employed instead of bone marrow in most cases of autologous transplantation [5]. Mobilization usually consists of a combination of both chemotherapy and leukocyte growth factors.

Furthermore, in the allogeneic setting, the number of transplantations with PBPC mobilized in healthy donors by GF alone is on the increase [6]. The main advantage of PBPC is faster recovery of hemopoiesis. Also, donors need not undergo general anesthesia.

Hemopoietic progenitor cells bear the CD34 antigen, and after *in vitro* cultivation they initiate colonies of more differentiated cells, e.g., colony-forming units for granulocytes and monocytes and burst forming units. The number of $CD34^+$ cells and CFU-GM are utilized in most transplant centers as a standard control of graft quality [7]. Using immunological methods with monoclonal antibodies, $CD34^+$ cells can be selected from the whole graft (positive selection). Using antibodies against certain unwanted cells (e.g., T cells or malignant cells) can eliminate them from the graft [8].

Classic myeloblative allogeneic transplantation of bone marrow or PBPC is still associated with substantial mortaliy and morbidity, usually due to infection and graft versus host disease. This is an unacceptable risk for the treatment of primarily non-malignant disease. However, there are new non-myeloablative regimens that are less toxic. If they become a standard approach, they will decrease morbidity and mortality in the allogeneic setting [5].

Autologous PBPC transplantation in the treatment of autoimmune diseases

Autologous hemopoietic cell transplantation following high dose chemotherapy and/or total body irradiation is a well-established and technically feasible procedure that has low mortality and morbidity, especially when using PBPC. Most centers report a procedure-related mortality of less than 5%. Therefore, high dose chemo/radiotherapy with autologous PBPC support is preferred for trials of immunoablative treatment in autoimmune diseases [3].

In animal models, after autologous and syngeneic bone marrow transplantation, some lymphocyte populations re-undergo ontogeny, and new self-tolerance can be induced by re-education. Thus, it is not only prolonged deep immunosuppression that is responsible for the decreased activity of the autoimmune disease in these models [9]. In humans,

PBPC = peripheral blood progenitor cells
GF = growth factor

CFU-GM = colony-forming units for granulocytes and macrophages

following autologous bone marrow or PBPC transplantation, different lymphocytes undergo reconstitution depending on the subset to which they belong. Most data show that B cells completely recapitulate ontogeny within 12 months [10,11]. On the other hand, T cell reconstitution from an unpurged graft is not considered as a recapitulation of ontogeny. In addition, T cells reconstitute more rapidly after PBPC than after bone marrow transplantation [12]. Memory $CD4^+45RO^+$ T cells develop mostly from the lymphocytes present in the graft, as do $CD8^+$ cells. They reconstitute their function within 2–3 months. However, naive $CD4^+45RA^+$ cells reconstitute with significant delay (6–12 months and longer), mostly due to lack of thymic maturation [13]. Thus the theory of tolerance induction by re-education of potentially autoreactive cells in the human autologous setting remains controversial, at least in unpurged, i.e., non-T cell-depleted grafts. Prolonged immune reconstitution is considered to be responsible for most of the effect of high dose immunosuppression with PBPC support in human autoimmune diseases.

Immunosuppression and immunomodulation represent a strategy in the management of most diseases with an autoimmune mechanism. Certain forms of these diseases do not respond even to new immunomodulating agents or more intensive immunosuppression, or they require a higher dose of chronic immunosuppression. Experiments in animal models of these diseases as well as initial clinical experience show that in such intractable disease forms, immunoablative doses of chemotherapy, which usually require autologous hemopoietic stem cell support to reduce inevitable myelotoxicity, can be effective [14]. The diseases studied primarily include multiple sclerosis, systemic sclerosis, systemic lupus erythematosus, juvenile arthritis and rheumatoid arthritis.

Multiple sclerosis and immunosuppressive therapy

MS is an inflammatory autoimmune disease of the central nervous system. Auto-reactive $CD4^+$ T lymphocytes cross the blood-brain barrier and induce a complex inflammatory reaction, perivenular infiltrates and, finally, the localized destruction of myelin [15]. The immune destruction of myelin also involves pro-inflammatory cytokines (tumor necrosis factor alpha and beta), immunoglobulins, complement, proteases, oxygen-reactive metabolites and nitric oxide [16]. Dysregulation of the cytokine network, the dominant activation of Th 1 cells, and insufficiency of suppressor cells all affect the course of the disease. During an attack, inflammatory activity is increased, and a greater number of T cells, memory cells, $CD57^+$ cells and $CD4^+28^+$ cells can be detected in the cerebrospinal fluid. Similar changes can be found in the peripheral blood, including functional changes of suppressor cells. Production of interferon-gamma and TNF-α is increased during the attack and, in many cases, can predict its onset. Analysis of T cell subpopulations and their activation can also detect the subclinical activation of MS and is more consistent with findings on magnetic resonance imaging [17].

Experimental allergic encephalomyelitis is the animal model of MS that can be induced in mice and rats by exposing them to encephalitogenic antigens. MS can have a relatively benign course, with an inflammatory process of low activity and sufficient reparative ability of oligodendrocytes. On the other hand, it can pursue a rapid malignant course with high inflammatory activity, oligodendrocyte destruction and no signs of reparation. In 80–90% of patients, MS initially has a remittent course, subsequently followed by a secondary progressive phase. In the remaining patients, the course is characterized from the onset by a gradual increase of disability without remissions (primary progressive form). Clinical symptoms of MS depend on the localization of myelin destruction. Typical signs include impairment of the optic nerve, oculomotoric disability, impairment of different sensitive pathways, cerebellar and movement disorders, sphincter disorders, and neuropsychological impairment. In more advanced stages, there is marked involvement of the lower extremities. This represents the base for the evaluation of disability according to Kurtzke´s Expanded Disability Status Scale. In this scale, from 0 to 10, 0 represents no neurological deficit and 10 signifies death from MS. Another scale, Scrips Neurological Rating Scale, reflects mainly upper extremity disability and deficits of cognitive functions [18,19].

Current therapeutic modalities distinguish between the treatment of attacks (high doses of corticosteroids IV) and therapy during the intervening periods. The latter is represented by long-term costly therapy with IFN-β, or combined immunosuppression, e.g., cyclophosphamide, methotrexate and azathioprine. Most patients in advanced stages of the disease are also on corticosteroids, which are given in higher doses to patients with rapidly progressive disease. In cases of very rapid neurological deficit progression, pulse therapy with IV steroids is indicated, or salvage therapy with second-line cytotoxic drugs: IV cyclophosphamide or mitoxantrone [20,21]. Patients who do not respond to salvage pulse therapy have a very poor prognosis. For those who progress to a stage of being completely confined to a wheelchair, the 10 year survival is a mere 30% [22]. Their prognosis thus approaches that of malignant diseases. Patients with rapid progression in disability, i.e., a gain of at least 1.0 point on the Kurtzke scale in the previous year, but without lower extremity paraplegia and with a preserved ability to walk at least several meters with a cane, may be candidates for trials of high dose immunosuppression with hemopoietic stem cell rescue.

Profound immunosuppression, on the level of ablation of the immune system, and its replacement with a new one (allogeneic transplantation) or with immature hemopoietic

MS = multiple sclerosis
TNF-α = tumor necrosis factor α
IFN-β = interferon-beta

progenitors can finally limit the dectructive process. This is based on three levels of evidence:

- Experiments on animal models with EAE.
- Anecdotal reports of disability stabilization or improvement in patients with MS undergoing high dose chemo/radiotherapy with hemopoietic stem cell transplantation for coincidental malignant disease [23,24].
- Results of first phase I/II clinical studies on high dose immunoablative therapy in patients transplanted primarily for MS.

Bone marrow transplantation in the treatment of EAE

Experimental allergic encephalomyelitis induced in susceptible strains of mice and rats is a well-established model of human MS. It is considered to be a Th1 lymphocyte-driven autoimmune disease. High dose cyclophosphamide or TBI with subsequent syngeneic bone marrow transplantation can induce remission of EAE in SJL/J mice [25]. Similar results were achieved in BUF rats with EAE with 6 to 33% spontaneous relapses. Moreover, 12–44% of animals relapsed after re-exposure to the inducing antigen [26].

In this experiment, a temporary impairment in neurologic disability in association with TBI was reported. The fact that more than one-half of the animals achieved long-term remission and/or resistence to reinduction after full immunological recovery supports the theory of tolerance induction after lymphocyte ontogeny recapitulation following syngeneic, pseudoautologous and autologous transplantation in animals. A high rate of permanent remissions and fewer spontaneous and induced relapses were reported by the same investigators in BUF rats with EAE treated with allogeneic bone marow transplantation. In this experiment, relapses were somewhat more frequent in animals that did not achieve full chimerism. On the other hand, relapses were rare in animals with chronic GVHD.

Syngeneic bone marrow transplantation after TBI in Lewis rats with EAE resulted in remission and in the clearance of autoreactive T lymphocyte clones characterized by Vbeta chain T cell receptor restriction from the central nervous system [27]. The investigators pointed out the influence of disease stage on the outcome of immunoablative therapy in the same type of transplantation. When the procedure was done early in the course, before glial scars formed, the effect was better [28].

Clinical experience with hemopoietic cell support in MS

MS represents the largest group of autoimmune diseases specifically referred for high dose immunosuppression with autologous stem cell support. To date, 48 transplanted patients have been described (case reports or results of phase I/II studies), either in the form of abstracts or full articles in the literature [Table 1].

First, 15 patients with both primary and secondary progressive forms of MS were treated in Thessaloniki, Greece [29]. In all, there was an improvement in the SNRS, and seven patients also improved their Kurtzke (EDSS) scores. Relapses occurred in two patients on the SNRS. No patient relapsed on the EDSS. Conditioning involved the combination of cytotoxic agents well known from autologous transplantation for malignant lymphomas: BCNU, Etoposide, ARA-C and Melphalan (BEAM). This regimen is extremely myelo- and lymphotoxic, and relatively less toxic to extrahemopoietic organs and tissues, with minimal neurotoxicity. On the first and second day after PBPC reinfusion (day +1 and day +2) IV anti-thymocyte globulin was administered, as a form of *in vivo* T cell depletion. *Ex vivo* purging was not performed. There were no serious or life-threatening complications. The next nine patients were treated by the same investigators using the combination BEAM and anti-thymocyte globulin, but additional *in vitro* T cell depletion of the graft was performed. One patient died of invasive aspergillosis, and one patient developed thrombocytic thrombocytopenic purpura requiring exchange plasmapheresis [30].

The Chicago group published results of an immunoablative regimen consisting of TBI, cyclophsophamide and methylpredinsolone in six patients [31]. In all, the autolo-

Table 1. Autologous transplantation for intractable multiple sclerosis

	No. of patients	Conditioning	*In vitro* T cell depletion	Fatal complications	Effect on disability
Thessaloniki [29]	15	BEAM + ATG	No	0	7 x improvement 8 x stabilization
Thessaloniki [30]	9	BEAM + ATG	Yes	1 x invasive aspergillosis	Short follow-up
Prague [32]	8	BEAM	7 x yes	0	3 x improvement 5 x stabilization
Chicago [31]	6	TBI,Cy,MP	Yes	0	3 x improvement 3 x stabilization
City of Hope [33]	5	Bu,Cy,ATG	Yes	1x influenza A	Short follow-up

BEAM = BCNU, etoposide, cytosinarabinoside, melphalan; ATG = anti-thymocyte globulin, Cy = cyclophosphamide, MP = methylpredisolone, Bu = busufan

EAE = experimental autoimmune encephalomyelitis
TBI = total body irradiation
GVHD = graft vs. host disease
SNRS = Scrips Neurological Rating Scale

gous graft was purged *ex vivo* from T cells. The regimen was very well tolerated with no signs of direct neurotoxicity and no life-threatening complications. All six patients demonstrated subjective and objective improvement. Half the patients had advanced-stage disease with paraplegia and were confined to a wheelchair. Therefore, the objective improvement in these patients could not be transposed on the Kurtzke EDSS; however, they did improve their SNRS. The remaining three patients improved both their EDSS and SNRS scores. No new CNS lesions were noted on MRI during follow up (5–17 months).

Recently, our group published the results of eight transplanted patients with secondary progressive MS. The BEAM combination was used as conditioning. Toxicity of the procedure was manageable and was not associated with serious complications. Both subjective and objective improvement occurred in six patients, and EDSS improved in three. An important observation was the improvement in quality of life, associated with the tapering of chronic immunosuppressive therapy in most patients after the procedure [32].

A group of five patients with MS was treated by high dose chemotherapy and autologous stem cell support in City of Hope, California, USA [33]. In two cases a flare-up of the disease occurred during PBPC mobilization with granulocyte colony-stimulating factor alone. In four cases, a combination of high dose busulfan, cyclophosphamide and anti-thymocyte globulin was used as conditioning; in one case the BEAM regimen was used. One patient died of type A influenza after the procedure. The relatively short follow-up did not allow the investigators to draw any conclusion in terms of effect on neurological disability.

Anecdotal case reports of high dose immunosuppressive therapy with stem cell support in MS have also been reported [34,35]. At this point, there are no data on MS treated primarily with high dose immunosuppression and allogeneic stem cell support. Several phase II clinical studies of high dose immunoablative therapy with autologous stem cell rescue are presently underway. Results are reported to the joint European League against Rheumatism (EULAR) and the European Group for Blood and Marrow Transplantation (EBMT) registry [14].

Summary and perspectives

Based on results of initial phase I/II clinical studies, we can state that high dose immunosuppression with autologous hemopoietic stem cell transplantation is a feasible procedure with acceptable toxicity. The clinical effect on neurological disability is promising in terms of stabilization of advanced and rapidly progressive disease; also, some improvement was observed even in heavily disabled patients. However, the follow-up of most patients is still too short for us to reach any definite conclusion.

Several questions remain unanswered regarding the technical approach and details of high dose immunosuppressive therapy. The need for T cell depletion is one such question. Both autoreactive T lymphocytes contaminating the graft and those that survive the conditioning regimen *in vivo* are involved in relapses of autoimmune diseases in animal models [36]. Early relapses after unmanipulated autologous stem cell transplantation in humans support this theory [37]. In fact, most centers that practice high dose immunosuppressive therapy tend to perform some form of T cell depletion. However, early relapses of diferent autoimmune diseases after autologous transplantation with T cell-depleted grafts have also been reported [38,39]. Results of ongoing studies should clarify this dilemma.

Another important issue discussed in this field is the role of new non-myeloablative conditioning regimens and allogeneic donor stem cell transplantation. The replacement of a damaged immune system by a new one from a healthy individual is associated with better outcome in animal models. Non-myeloablative regimens are also considerably less toxic than classical allogeneic transplantation. Initial results in the treatment of hematological malignancies are encouraging and attractive for the treatment of intractable autoimmune diseases [4].

Immunoablative conditioning that does not require stem cell rescue is another possible direction for future treatment of autoimmune diseases, including MS. Results with high dose cyclophosphamide are promising; they may support the theory that profound immunosuppression is the only factor responsible for the effect of high dose immunoablative treatment with stem cell rescue [40]. This approach eliminates the danger of reinfusing autoreactive lymphocytes with the autologous graft and should be further explored. However, it must be taken into account that sensitivity of bone marrow to high dose cyclophosphamide may be individual, with the risk of protracted cytopenia requiring long administration of leukocyte growth factors, thus increasing the risk of disease flare-up.

If the results of ongoing studies of immunoablative treatment with stem cell support confirm their clinical benefit in advanced stages of the disease, a shift of this method to earlier disease stages will be feasible. In that case, the role of maintenance therapy using immunomodulatory agents after the procedure should also be explored.

EDSS = Expanded Disability Status Scale
MRI = magnetic resonance imaging

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