Artigo Especial / Special Article

**Stem cell therapy for severe autoimmune diseases**

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Intense immunosuppression followed by allogenic or autogenic hematopoietic stem cell transplantation is a relatively recent procedure which was used for the first time in severe, refractory cases of systemic lupus erythematosus. Currently three aggressive procedures are used in the treatment of autoimmune diseases: high dose chemotherapy without stem cell rescue, intense immunosuppression with subsequent infusion of the allogenic hematopoietic stem cell transplantation combined with or without the selection of CD34+ cells, and the autogenic hematopoietic stem cell transplantation. Proof of the graft-versus-leukemia effect observed define SCT as a form of immunotherapy, with additional evidence of a similar Graft-vs-Autoimmunity effect which is suggestive of a cure for autoimmune diseases in this type of therapy. The use of allogenic SCT improved due to its safety compared to autogenic transplantations. In this report, data of multiple sclerosis and systemic lupus erythematosus are reported, with the conclusion that Immunoablation followed by SCT is clearly indicated in such cases.

**Keywords**: Hematopoietic stem cell transplantation, autoimmune diseases, multiple sclerosis, systemic lupus erythematosus

**Introduction**

The interest in autoimmune diseases (ADs) is increasing exponentially (1, 2). Their estimated prevalence in Western countries ranges from 3% to 70% of the general population (3). Autoimmunity coincides with the loss of tolerance to the self (4); it is thought of as a persistent failure of an integrated fabric of components rather than the adverse consequence of a specific “forbidden clone” (5). It is not known whether the antibody response in systemic ADs is antigen-driven, such that the immune system is responding to self-proteins that have become autoantigenic (6), or if ADs represent a primary dysfunction of the immune system (7). The two hypotheses are not mutually exclusive and the prevailing conception is that of a combination of genetic factors responding to environmental triggers (8), the latter including both exogenous and endogenous factors. The majority of ADs are controlled, more or less satisfactorily, by conventional therapeutic manipulation of the immune system, but there is a hard core of refractory/relapsing, treatment-resistant (9) ADs for which the term “malignant autoimmunity” has appropriately been proposed (10).

Intense immunosuppression (“immunoablation”), followed by allogeneic or autologous hemolymphopoietic stem cell (HSC) transplantation, is a relatively new therapeutic approach, which was...
proposed for the first time in the clinic for the treatment of severe, refractory systemic lupus erythematosus (SLE) (11). Immunoablation has produced encouraging results in patients with ADs who have undergone allogeneic bone marrow transplantation because of coincidental hematologic malignancies (lit. in 12-13). A great deal of prior research had already produced impressive results using transplant-based procedures in experimental animals. Suggestions to carry these encouraging results into the clinic soon followed (14-16).

There are now three new aggressive approaches for the treatment of severe autoimmune diseases (SADs) of the refractory (relapsing) life-threatening subtype. High-dose cyclophosphamide with no stem cell rescue has been attended by encouraging results in the John Hopkins single center experience (17). However intense immunosuppression is most generally followed by the infusion of hematopoietic stem and progenitor cells included in the CD34 selected compartment. Autologous HSCT, which originated from the classical van Bekkum’s (18) and Ikehara’s (19) animal experiments, are being utilized worldwide because of the procedure’s greater safety, although transplant-related mortality (TRM) has been unexpectedly high (20). Patients with multiple organ insufficiency because of the ubiquitous damage inflicted by advanced systemic autoimmune diseases appear to be at greatest risk of TRM. The more intense immune suppressive preparative regimens when combined with CD34+ selection of the graft have also been associated with lethal opportunistic infections (21).

It is still uncertain whether the mechanism of action is essentially immunosuppressive, or whether lymphoid reconstitution following mobilization plus conditioning may ensure the emergence of a tolerant immune system vis-à-vis of the same autoantigens that had driven the autoimmune process (4). Be that as it may, clinical results are encouraging and even dramatic in properly selected patients. Some of the best results are being obtained in SLE, as will be discussed later, and in active progressive MS, where the abrogation of all gadolinium enhancing lesions has been found in Genoa (22). From the initial Phase I/II clinical studies, randomized phase III trials are currently evolving in Europe, including the ASTIS (SSc), ASTIMS (MS) and ASTIRA (RA) and in America as NIH funded trials for MS, SLE, and scleroderma.

The pertinent literature is copious, and a number of exhaustive reviews of the experimental (18, 19) and clinical (14, 16, 23) aspects of these therapeutic approaches have been published recently. No effort will be made here to examine in depth the pre-clinical area, which has been discussed elsewhere (19).

**Can autoimmune diseases be cured?**

Without wishing to enter into the new areas of biological treatment of autoimmune rheumatic diseases (24), there are two fundamental requirements to restore tolerance to an immune system that may be fluctuating between autoimmunity and tolerance (4), but in severe refractory autoimmunity diseases (SADs) is markedly shifted away from tolerance. Stem cell transplantation procedures aim to remove an immune system harboring cohorts of lymphoid subpopulations irreversibly sensitized to “self” epitopes/antigens. This may be obtained following SCT, whether allogeneic, syngeneic and autologus in a decreasing order of efficacy.

**Evidence for a clinical Graft-vs-Autoimmunity effect**

One of the most important findings in allogeneic BMT for leukemic and other malignancies, both in animal experiments and in human disease, is the well-known Graft-vs-
Leukemia (GVL) effect. As stated recently, this GVL effect "is very real, and thousands of patients are alive because of it" (26). In this line of thought SCT has been defined as a form of immunotherapy (27) evidence is now accumulating both in experimental and clinical settings, that a similar Graft-vs-Autoimmunity (GVA) effect might also exist, consisting in the substitution of normal T, B and lymphoid progenitor cells in the place of an autoimmune lymphoid system (28). The GVA effect is supported by experiments showing that mixed chimerism obtained utilizing a sublethal irradiation conditioning regimen followed by allogeneic BMT can prevent the onset of diabetes and even reverse pre-existing autoimmune insulitis in nonobese diabetic (NOD) mice, whereas the same radiation protocol without allogeneic SCT is insufficient (29).

A similar effect has been shown using sublethal conditioning and an anti-CD154 monoclonal antibody (30). It is clear that such an approach becomes critical when utilizing nonmyeloablative procedures (NST). Some recent observations and studies are showing that this GVA effect may be active also in the clinic. In a meta-analysis performed on 30 patients with coincidental diseases (AID plus a hematologic disease requiring allo-SCT; 31) it was found that 19 developed GVHD and 11 did not. Upon clinically justified discontinuation of all immunosuppressive therapy, 3 out of 11 patients without GVHD relapsed with their concomitant AID (freedom of AID-relapse 69% at 9 years), whereas none of the 19 patients with GVHD did so (p=0.0135). In addition, freedom of AID relapse was superior following allo-rather than auto-SCT (89% at 18 years vs 35% at 4.5 years; p=0.0001).

There are also single but suggestive case reports. A child with thalassemia intermedia developed an autoimmune hemolytic anemia (AIHA) severe enough to promote an auto-SCT, had a short and incomplete remission, relapsed with severe hemolysis, and finally was cured following allo-BMT from an unrelated volunteer donor (VUD; 32.). This may well be the first clinical demonstration of a superior curative potential of allo-vs-auto-SCT.

A non myeloablative allogeneic PB SCT was given to a patient with CML in first chronic phase who was also suffering with severe systemic psoriasis and psoriatic polyarthritis (33). After complete remission of both diseases there was a relapse coincident with the increase of host DNA, but finally discontinuation of CSA was followed by complete chimerism and remission of both diseases.

There are now also three fully published cases of patients suffering with Evans'ssyndrome that obtained CR following allo-SCT (34) In the case by Oyama et al (35) CR occurred only after grade IV GVHD had appeared following discontinuation of immunosuppressive therapy because of intolerance. Even more demonstrative is a case of ours in which an allo-BMT from an HLA-identical sister was followed by relapse and mixed chimerism evolving towards rejection, and in which complete donor chimerism and a clinical-biologic CR followed a series of 5 gradually incremental DLI, the last one associated with grade II GVHD (36).

**Autologous Transplants for the Treatment of Autoimmune Disease**

Autologous HSC transplants (ASCT), from marrow or now almost exclusively from peripheral blood, are much more commonly used to treat ADs than are allogeneic transplants for two reasons: the encouraging experimental results from Rotterdam (37, 38) and from Jerusalem (39), and the greater safety of the autologous procedures. TRM at 2 years post-transplant for ADs was 8.6 %, which is comparable to the procedure-related mortality following transplantation for non-Hodgkin’s lymphoma (NHL) (40). Contributing factors to a higher than expected TRM may have been a learning curve for utilizing ASCT in new diseases, hitherto unrecognized hazard associated with profound immunodeficiency, especially following intense T-cell depletion, and unique organ dysfunctions, as heart and lung failure in systemic sclerosis (41).

Exhaustive reviews have been published recently (14, 16, 23), in addition to multicenter retrospective studies (42). Only a synthesis of the Genoa results will be reported here.
Multiple Sclerosis (MS)

MS is characterized by demyelination, immunophlogistic lesions around axons, and ultimately axon loss. Pathogenesis is widely held as autoimmune (43, 44), with T-cell activity in the foreground (45). It has become the most common disease treated by ASCT, mostly because of extensive work pioneered by Fassas (46).

In a cooperative ongoing study 19 cases of secondary progressive MS with EDSS initially between 5 and 6, a documented rapid progression over the last year unresponsive to conventional therapies and the presence of Gd-enhancing areas on brain MRI using a triple dose of Gd (47) underwent CD 34+ mobilization and then ASCT following conditioning with BEAM (48). Eighteen cases have undergone ASCT with a median follow-up of 15 months (range 6-36 months). No major serious adverse events were observed during and after treatment. Mobilization was successful in all cases, with a median number of 9.06x10^6/kg of CD34+ collected.

During the 3-months pre-treatment period 346 Gd-enhancing areas/month/patient had appeared. The number of Gd-positive areas decreased dramatically already after mobilization with CY and dropped to 0 within one month from conditioning with BEAM. All patients slightly improved clinically, or remained stable. The median EDSS decreased to 6 and the median Scripps scale increased to 70. In the first case MRI enhancing was still completely abrogated 36 months after transplantation. Although clinical amelioration/stabilization were observed, it was concluded that the final impact of this procedure on the natural history of the disease remains to be established in larger, possibly prospective randomized trials.

Systemic lupus erythematosus

It is well known that survival in SLE has improved dramatically, so much so that care is gradually shifting from the treatment of immunophlogistic lupus to the one of atherosclerotic complications (49). At the pinnacle of the lupus iceberg, however, there are still cases of refractory-relapsing disease. For such patients I proposed HSC transplantation in 1993 (50). Despite the paradox of trying to restore tolerance by transplanting the patient’s own HSC, the greater safety of the autologous procedure, buttressed by the experiments discussed above, induced us to transplant the first SLE patient with her own T-cell depleted (3 log) marrow in 1996 (51). This is the SLE patient with the longest follow-up in the literature. After 3 years of complete, corticosteroid-free remission there was a gradual reappearance first of ANA (first of the speckled subtype and later of the homogeneous), then of anti-dsDNA (Crithidia), and finally of mild to moderate proteinuria, which is being successfully treated with a combination of low-dose corticosteroids and mycophenolate mophetil (MMF). Another patient with class IV WHO glomerulonephritis, severe nephrotic syndrome and massive proteinuria (>20 g/24h) has undergone transplantation with autologous mobilized HSC, and has had a dramatic remission, followed by a benign, responsive relapse (28).

Leaving aside 4 cases of coincidental SLE-malignancy (lit in 14, 16), there are 6 fully published case reports of nonconcomitant SLE patients having undergone autologous peripheral blood HSC transplantation (52-55). All patients attained complete remissions, but in most of them there was a biological relapse (ANA positivity) after 2-3 years from transplant. In the most extensive single centre clinical study published to date (56) 9 patients underwent stem-cell mobilization with CY 2 g/m^2 and G-CSF 10 mg/kg. Two patients were excluded from transplantation because of infection (one death from disseminated mucormycosis), and 7 were autotransplanted after conditioning with CY (200 mg/kg), 1g methylprednisolone, and 90 mg/kg equine antithymocyte globulin. All patients were seriously ill, with SLE disease activity indices (SLEDAI) of 17-37, including 1 case with alveolar hemorrhage and 4 with WHO class III-IV glomerulonephritis and nephrotic syndrome. Lupus remained in clinical remission in all patients after transplant. ANA became negative, and spontaneous T-cell activation marker CD69 declined or normalised after transplantation.
Retrospective multicentric studies are being currently performed under the auspices of the EBMT/EULAR in Europe and the ABMTR in the U.S. The unavoidable shortcomings of such studies are known, but some indications may surface. There is still uncertainty as to the real role of ASCT. Two interpretations are possible. It has been proposed that the conditioning, inclusive of ATG, might provide “a window of time” free of memory T cell influence, during which the maturation of new lymphocyte progenitors may occur without recruitment to anti-self reactivity (56). On the other hand one may suspect that the transplant’s immunologic effect mainly resides in a sort of “debulking” of the inflammatory-autoimmune load, as already pointed out (25, 28) and again recently considered in a clinical study of ASCT in 14 patients with RA (57). Not all pathogenic T lymphocytes appear to be eradicated, and related mechanisms may still be operative even after intensive immunosuppression and ASCT (58). In order to elucidate whether, if relapses occur, disease is reinitiated by lymphocytes surviving the conditioning regimens, or from the HSC compartment or from inciting autoantigens, studies are being performed with gene-marked autologous HSC (59). Immune reconstitution following ASCT has been studied extensively, and it is well-known that the most common immunologic feature is a severe, prolonged depression of CD4+ T cells. In our two transplanted SLE patients the depression of CD4+ and CD45 RA cells lasted for years, and did not hinder the reappearance of ANA. Anyway, from operational point of view, there is no doubt that therapeutic results can be dramatic.

The excessive TRM which has been found in a multicentric retrospective study may have many causes. Patients with severe, long standing connective tissue disease such as SSC and SLE have more multiorgan lesions and dysfunctions than patients with leukemia, who are generally transplanted early in their disease. In addition, it appears that high-intensity conditioning is associated with significantly higher 1-year TRM (21).

While the immediate and short-term therapeutic effects are evident, long term results are still awaited. In our almost 5 years old ASCT for SLE there was a very gradual progressive relapse from ANA to anti-ds ANA positivity, and hence to slight to moderate proteinuria. However this patient enjoyed 4 years of corticosteroid independence, which made of her quite another woman. A similar pattern was seen in a male patient with the nephrotic syndrome. A common feature of most relapsed patients is a restored responsiveness to low-intensity treatment, including MMF.

Finally, a competition is appearing between high-density immunosuppression alone (the Johns Hopkins regimen) and immunosuppression with HSC rescue. Both programs expose to the problem of late oncogenicity, which cannot be ignored, especially in young patients with nonmalignant disease. Some of these patients may have already been treated with alkylating agents, and this may be an additional risk factor for the development of myelodysplastic-leukemic (MDS/AML) disease. However, intense therapy can be life-saving. The difference in neutrophil and platelet recovery between the two programs is not that impressive, but this may change with a wider and better utilization of HSC expansion ex vivo (60, 61).

ASC transplantation is a powerful therapeutic measure for AID, including SLE. While achieving an authentical cure seems improbable at this time, prolonged, steroid-free remissions are a reality. The selection of patients may be troublesome, because the very refractory lesions which call for a powerful intervention may be the cause for severe toxicity. Total care and referral to centres of excellence are mandatory for these patients (62).

Conclusions

Immunoaablation followed by SCT is a powerful therapeutic procedure for SADs. Autologous SCT is a certainly safer and “easier” procedure, and results in certain diseases such as MS are outstanding. In other, more aggressive SADs such as autoimmune diseases of the blood, the intervention of a GVA effect has shown its efficacy. In such cases the utilization of NST procedures is clearly indicated.
A terapia com células precursoras hematopoéticas nas doenças autoimunes
Alberto M. Marmont

Resumo

A imunossupressão intensa seguida do transplante de células precursoras hematopoéticas (TCPH), alogênicas ou autogênicas é um procedimento relativamente recente e que foi utilizado pela primeira vez em casos dramáticos de lúpus eritematoso sistêmico. Atualmente três procedimentos agressivos são utilizados nas doenças autoimunes: Altas doses de quimioterapia sem o resgate de células precursoras, imunossupressão intensa com impulsação subsequente de células precursoras hematopoéticas alogênicas combinadas ou não a seleção de células CD34+, e o transplante autogênico de células precursoras hematopoéticas. A comprovação do efeito enxerto contra a leucemia observado e comprovado, define o TCPH como uma forma de imunoterapia, existindo evidencias também do efeito contra a imunidade o qual propiciaria a cura das doenças autoimunes nesta forma de terapia. O uso do TCPH autogênico teve seu avanço baseado na segurança do mesmo em relação aos TCPHs alogênicos. No relato são apresentados dados em esclerose múltipla e lúpus eritematoso sistêmico sendo nossa conclusão de que as TCPHs nas suas modalidades tem indicação a luz dos resultados na literatura.

Palavras-chave: Transplante de células precursoras hematopoéticas, doenças autoimunes, Lúpus Eritematoso Sistêmico, esclerose múltipla

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