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PRESENT AND FUTURE OF HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Unrelated bone marrow transplantation in thalassemia. The experience of the Italian Bone Marrow Transplant Group (GITMO)

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Background and Objectives. Allogeneic bone marrow transplantation (BMT) is a widely accepted therapeutic approach in homozygous β-thalassemia. However, the majority of patients do not have a genotypically identical donor within the family. This prompted us to conduct a pilot study to investigate the feasibility of matched unrelated bone marrow transplantation in thalassemia. The major drawback was the high risk of immunologic and transplant-related complications, mainly graft-versus-host disease (GvHD) and graft failure.

Design and Methods. Our aim was to reduce this risk through careful selection of donor/recipient pairs. HLA haplotypes that show a high linkage disequilibrium among their class I, class II and class III alleles are considered extended or ancestral haplotypes.

Results. These haplotypes are conserved and can be shared by apparently unrelated individuals. Our study shows that matching for these haplotypes significantly improves the outcome of unrelated bone marrow transplantation in thalassemia. In fact, results were comparable to those obtained in transplants using HLA-identical family donors.

Interpretation and Conclusions. Better results were obtained in patients with lesser iron overload and when the donor shared an identity for the DPB1 alleles.

Key words: unrelated BMT, thalassemia, extended haplotypes, DPB1 alleles.

Controversies and perspectives on the use of stem cells

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In Western countries, more than 70% of patients with homozygous β-thalassemia lack an HLA-identical family donor. Until recently this left them with no option other than life-long transfusion and iron-chelating therapy. Over the past ten years, there has been a steady increase in the number of bone marrow transplants (BMTs) from unrelated donors.[1] At first, results were unsatisfactory with a high risk of transplant-related complications, particularly acute and chronic graft-versus-host disease (GvHD) and graft failure. These complications are likely to be the consequence of HLA differences not revealed by previous HLA typing techniques.[2] The introduction of high resolution molecular techniques for histocompatibility testing has markedly improved the outcome of unrelated transplants with results comparable to those obtained in transplants using HLA-matched family donors.[3] An important challenge was to achieve similar results in thalassemic patients.

In HLA-matched unrelated individuals, the entire structure of an HLA extended or ancestral haplotype (EH) is generally identical except for rare variations at the centromeric and telomeric extremities. Two unrelated individuals who share two extended haplotypes are highly likely to be identical, not only for the routinely tested HLA class I, II and class III genes, but for the entire MHC region where there are many other genes that have an important role in antigen presentation and immune response.[4] Several mechanisms, including selection pressure, recombination suppression and preferential transmission, may explain the conservation and frequency of extended or ancestral haplotypes in different populations.[5] Based on the foregoing, we conducted a pilot study aimed at

exploring both the feasibility of BMT from a marrow unrelated donor (MUD) in thalassemia and the possibility of reducing the risk of immunologic complications by selecting donor/recipient pairs sharing extended haplotypes or parts of them.

Design and Methods

From November 1992 to February 2002, 43 patients with thalassemia major were enrolled into this study by six BMT Centers in Italy. Twenty of the patients were females and 23 males, their ages ranging from 2-28 years (median 14). Out of 43 patients examined, 19 were assigned to low and intermediate risk classes, and 24 to the high risk class. Alleles at the HLA-A, B, Cw, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1 and DPB1 loci were identified by PCR-SSP (Dynal, Oslo, Norway) and sequence-based typing. Amplification and sequencing of HLA class I and class II genes were performed using standard big dye terminator cycle-sequencing chemistry supplied with the ABI sequencing kit. Reactions were analyzed on an Applied Biosystem 310 Automated DNA sequencer. Alleles were assigned according to DNA sequences published by the *Nomenclature for the factors of HLA system* Committee.

In our study, HLA extended haplotypes were identified and defined by referring to the data provided by Rendine *et al.* and Contu *et al.* for the Italian population and to data from the 10th and 11th International Histocompatibility Workshops and the National Marrow Donor Program donor registry for other populations.[6-8]

Thirty-eight bone marrow donors were identified within the *Italian Bone Marrow Donor Registry*, another 3 were found in the *German National Bone Marrow Donor Registry*, one was found in the *French Bone Marrow Donor Registry*, and one in the *National Marrow Donor Program Donor Registry* of the USA. For 28 donors, at least one informative family member was typed for HLA haplotype deduction. In the remaining 15 cases, the haplotypes were assigned on the basis of the presence of at least one extended haplotype well-defined in the population. Six patients were transplanted after a preparative regimen including busulfan (BU) 14 mg/kg and cyclophosphamide (CY) 200/160/120 mg/kg. As 2 of these 6 patients did not have sustained engraftment, in the remaining 37 patients the conditioning regimen was modified as follows: BU 14, thiotepa (TT) 10 mg/kg and CY 200 for 17 patients (low and intermediate risk classes); BU 14, TT 10 and CY 160 for 6 patients aged less than 16 (high risk class); BU 14, TT 10 and CY 120 for 14 patients aged more than 16 years old (high risk class: adults). The median bone marrow nucleated cell dose was 3.6×10^8/kg of recipient weight (range 1.8-11.6). All patients received cyclosporine (CSP) and short-term methotrexate (MTX) for GvHD prophylaxis. Acute and chronic GvHD were graded according to the Seattle criteria. Chimerism was documented by *in situ* Y chromosome hybridization of either bone marrow or blood samples in sex-mismatched donor/recipient pairs, by analysis of variable number of tandem repeat (VNTR) polymorphisms and by microsatellite analysis of bone marrow and/or blood samples in the case of sex-matched pairs. For continuous variables with a symmetric distribution, the results are expressed as medians and ranges. Comparison between groups was performed by Fisher's exact test. Survival probability was estimated by the product-limit method of Kaplan and Meier.

Results and Discussion

Out of 43 donor/recipient pairs, 33 were completely identical for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1 and DQB1 loci. Seven pairs were completely identical for two extended haplotypes and 20 pairs shared one extended haplotype. Although the remaining 16 pairs did not share complete extended haplotypes, family segregation analysis performed in five cases in both donor and recipient showed haplotype identity in 3 cases. Only in one case was haplotype identity lacking.

In 30 cases (69.8%) the transplant was successful with complete allogeneic reconstitution. Five patients (11.6%) rejected the donor marrow and eight patients (18.6%) died from transplant-related complications.

None of the 7 recipients who shared two HLA EH and had sustained donor engraftment developed grade II-IV acute GvHD, while in the remaining 30 evaluable patients who shared either a single or no EH, the overall occurrence of acute GvHD was 52% (p=0.05). A significant reduction of the incidence of acute GvHD was observed in the group of patients who shared an identity for both DPB1 alleles with their donors, compared to in the group of patients who received bone marrow from a donor mismatched for either 1 or 2 DPB1 alleles (21% vs 61%, p =0.05). Moreover, the risk of acute GvHD was significantly increased (p = 0.01) in patients with an HLA class I minor-mismatch and one or two differences at the DPB1 locus compared to patients sharing at least one extended haplotype

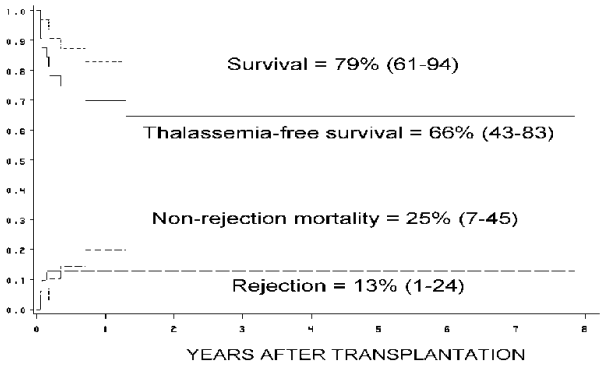

Figure 1. Kaplan-Meier probabilities of survival, thalassemia-free survival, non-rejection mortality and rejections for 41 thalassemia patients transplanted from HLA-matched unrelated donors (between parentheses, 95% confidence limits at two years).

and DPB1 identity with their donors (100% vs. 18%, respectively). In our series of 43 consecutive thalassemia patients, rejection and mortality rates were 11.6% and 18.6%, respectively. Sixty-nine percent of our patients are alive with sustained engraftment of donor hematopoiesis, this leading to a projected thalassemia-free survival of 66% (Figure 1). Overall survival and thalassemia-free survival (94.7% and 84.2%, respectively) in the 19 patients of class I and class II risk groups were comparable to those obtained in transplants from an HLA-identical family donor. The remarkable stability of the extended haplotypes[4] and the data deriving from MLC studies[9] suggest that two unrelated individuals sharing two HLA extended haplotypes are nearly always practically HLA-genoidentical, just as if they had inherited the HLA haplotypes from the same parents. Therefore, it is reasonable to hypothesize that the histocompatibility differences between a pair of HLA-genoidentical siblings and a pair of unrelated individuals sharing two extended haplotypes exclusively reside in minor histocompatibility antigens (mHAg) located outside the HLA region. Moreover, haplotype matching, even when it is not for complete extended haplotypes, makes it possible to include parts of them (telomeric or centromeric portion of extended haplotypes) that are common in populations worldwide.[10] The relatively low incidence of acute and chronic GvHD (44% and 28%, respectively) obtained in our study highlights the importance of a careful immunogenetic selection of donor-recipient pairs. So far, it is quite difficult to differentiate the role of DP[11] molecules from that of other HLA molecules as this would require the availability of genoidentical donor/recipient pairs different at the DP locus for a rare event of crossing-over. Alternatively, an optimal model for this type of evaluation is represented by unrelated donor/recipient pairs different at the DP locus but sharing two extended haplotypes. In our study, the incidence of immunologic complications was significantly reduced in DPB1-matched recipients.

Our results show that BMT from unrelated donors, especially when identical for at least one extended haplotype, may offer a probability of success comparable to that offered by transplants using HLA-identical family donors. It is, therefore, reasonable to consider this type of transplant as an acceptable therapeutic approach in thalassemia, at least for patients who are not fully compliant with conventional treatment and do not yet show irreversible severe complications of iron overload, provided that a careful immunogenetic selection of marrow donors is made.

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