Graft rejection after unrelated donor hematopoietic stem cell transplantation for thalassemia is associated with nonpermissive HLA-DPB1 disparity in host-versus-graft direction

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The success of allogeneic hematopoietic stem cell transplantation (HSCT) from matched unrelated donors (UDs) for β-thalassemia may be hampered by the occurrence of graft rejection. Here, we show that the rate of this complication can be reduced by selecting 5-loci HLA-matched donors without nonpermissive mismatches at HLA-DPB1, defined according to an algorithm previously described and based on principles of central T-cell tolerance. Seventy-two consecutive patients and their UDs, prospectively selected for matching at the allelic level for HLA-A, -B, -C, -DRB, and -DQB1 loci, were enrolled in the analysis. These pairs were either DPB1 matched/permissively mismatched (n = 45, control group) or had at least one nonpermissive DPB1 mismatch in the host-versus-graft (HvG; n = 17) or in the graft-versus-host (GvH; n = 10) direction. In multivariate analysis, the risk of rejection was significantly increased in the group with HvG disparity (RR = 7.42; 95% CI = 1.29-42.68; *P* = .02) as compared to the control group. A lower, statistically significant, probability of thalassemia-free survival was found in patients belonging to the HvG group as compared to controls (RR = 5.15; 95% CI = 1.58-16.82; *P* = .01). These data suggest that in patients with thalassemia, the incidence of graft failure after HSCT may be reduced by appropriate selection of UDs, with such selection taking into account the functional rules of immunogenetics. (Blood. 2006;107:2984-2992)

Introduction

β-thalassemia is an inherited disorder of hemoglobin synthesis that is associated with a reduced quality of life due to dependency on continuous blood transfusions and the need for lifelong administration of iron-chelating agents. Moreover, this disorder results in elevated social and economic costs and, especially in patients with poor compliance to iron chelation therapy, in reduced life expectancy, mainly due to complications related to iron overload and, less frequently, to viral infections acquired via blood transfusions.[1,2]

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only definitive cure for this disease,[3-7] which represents the most frequent inborn genetic disorder in the Mediterranean countries.[1] One impediment to the success of allogeneic HSCT for the cure of β-thalassemia is represented by graft rejection, whose incidence is considerably higher than that observed in patients receiving transplants for leukemia.[7-9] Several factors may contribute to the occurrence of this complication, including avoidance of any immunosuppressive or myeloablative treatment before transplantation, possible alloimmunization related to erythrocyte transfusions and, especially in transplantation using unrelated donors (UDs), disparity for major or minor histocompatibility antigens. Interestingly, in thalassemia patients receiving a transplant from an HLA-identical sibling, the incidence of rejection is directly correlated with the patient's class of risk, defined according to the Pesaro criteria, an incidence of 4%, 8%, and 12% being observed in patients belonging to risk class 1, 2, or 3, respectively.[8,9]

Fewer than 25% of thalassemia patients have a nonaffected HLA-matched sibling donor; for undergoing transplantation, the remaining patients need to find a suitable, HLA-compatible donor within the registries of unrelated volunteers now enrolling more than 9 million donors worldwide.[10,11] To avoid the occurrence of life-threatening or invalidating immune-mediated complications in patients with a nonmalignant disorder, matching of UD-recipient pairs must be based on high-resolution typing for HLA-A, -B, -C, -DRB, and -DQB1 loci. Only 20% of UD-recipient pairs matched

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for these loci are also compatible for HLA-DPB1, due to the very weak linkage disequilibrium existing between the DR/DQ loci and the DP locus. Consequently, more than 80% of UD transplantations are performed across the HLA-DPB1 barrier.[12] We have previously proposed an algorithm for the determination of nonpermissive HLA-DPB1 disparities, which were found to be associated with a significantly increased risk of transplant-related mortality (TRM) and of grade II-IV acute graft-versus-host disease (aGvHD), in patients undergoing transplantation for malignant hematopoietic disorders.[13] The algorithm is based on the identification of an immunogenic T-cell epitope shared by a defined subset of HLA-DPB1 alleles, which, if expressed by self–HLA-DP molecules, protects from mounting a response against allogeneic HLA-DP antigens carrying the epitope, due to thymic deletion of self-reactive T cells, but conveys susceptibility for becoming the target of such a response.[14]

In the present study, we demonstrate that the risk of rejection in UD HSCT for β-thalassemia is associated with the presence of nonpermissive HLA-DPB1 mismatches in the host-versus-graft (HvG) direction, as defined by our algorithm, regardless of the patient's class of risk. These results, indicating that immunogenetic factors play a crucial role for the occurrence of complications due to alloreactivity in UD HSCT for β-thalassemia, are potentially useful for selecting the most suitable unrelated volunteer.

Patients, materials, and methods

Patients and transplant characteristics

Seventy-two consecutive patients with thalassemia major, receiving a transplant from a UD between 1992 and 2004 at the Pavia, Cagliari, Bologna, and Pesaro centers, were included in this analysis. The study received approval by the local Institutional Review Board of each participating center and informed consent, according to the Declaration of Helsinki, was obtained from all patients or from their parents or legal guardians. The clinical characteristics of donor-recipient pairs are detailed in Table 1. Median time of follow-up for surviving patients is 24 months (range, 5 months to 12 years). All patients received transplants from a UD selected for complete matching by 4-digit sequence-based typing[15] or sequence-specific polymerase chain reaction PCR (PCR-SSP)[16] at the HLA-A, -B, -C, -DRB, and -DQB1 loci. Four-digit HLA-DPB1 typing was prospectively performed by PCR-SSP and by PCR–sequence-specific oligonucleotide probing (PCR-SSOP).[13]

Three different types of conditioning regimen were used in this cohort of patients. One subgroup of patients received the classic combination of busulfan (BU) and cyclophosphamide (CY), whereas the remaining 2 subgroups of patients were given a modified conditioning regimen, either adding thiotepa (TT) to the same BU-CY combination, or using a myeloablative therapy based on the use of oral BU, TT, and fludarabine (FLU). The 18 patients receiving the BU-TT-FLU conditioning regimen were also given antithymocyte globulin (ATG, 3.5 mg/kg) on days −3 and −2 (Table 1 provides further details). To prevent any risk related to persistent cytopenia in patients with poor graft function, an autologous rescue of bone marrow cells was harvested and cryopreserved before transplantation for all patients.

All patients received unmanipulated bone marrow cells. Marrow was infused after 36 and 72 hours following the last dose of CY and FLU, respectively.

All patients received cyclosporine A (Cs-A), 3 mg/kg/d intravenously, starting from day −2, and short-term methotrexate (MTX, 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3, +6, +11) for GvHD prophylaxis. Cs-A was switched to 6 mg/kg/d orally as soon as oral administration could be tolerated; starting from day +90, the dose was tapered, until discontinuation at 1 year.

Table 1. Clinical data for patients with β-thalassemia and their UDs

	HLA-DPB1*		
	Matched and permissive	Nonpermissive	
		HvG direction	GvH direction
No. of patients	45	17	10
Sex of donor/recipient, no. cases			
Female/male	11	3	3
Other combinations	34	14	7
Median age, y (range)			
Donor	32 (21-44)	33 (19-48)	36 (20-52)
Patient	15 (6-24)	15 (1.5-37)	14 (1.5-26)
Class†, no.			
1	14	5	2
2	12	6	2
3	19	6	6
Donor/recipient HCMV serology, no. cases			
Negative/negative	2	4	1
Other combinations	43	13	9
Median cell dose, $\times 10^8$/kg (range)	3.8 (2-8.3)	4.8 (0.2-15)	4.5 (0.3-12)
Conditioning, no.			
BU-CY	11	5	2
BU-TT-CY	21	9	6
BU-TT-FLU-ATG	13	3	2
Rejection, no.	2	4	1
aGvHD II–IV‡, no.	14	8	4
OS, no.	37	13	7
TFS, no.	35	10	6

*Classification of HLA-DPB1 alleles was performed according to the algorithm described before.[12]

†Class according to Pesaro classification.[8]

‡Seventy-one of 72 pairs were evaluable for aGvHD (45 matched or permissive, 9 nonpermissive in GvH and 17 nonpermissive in HvG direction).

Supportive therapy, as well as prophylaxis and treatment of infections, was homogeneous among participating centers. Reactivation of human cytomegalovirus (HCMV) was monitored either by expression of the pp56 antigen or by quantitative PCR and was treated with either ganciclovir or foscarnet on a preemptive basis.[17]

Definitions

Engraftment was documented by in situ Y chromosome hybridization of bone marrow or blood samples in sex-mismatched donor-recipient pairs and by analysis of variable number of tandem repeat (VNTR) polymorphisms on bone marrow or blood samples. Analysis of chimerism was performed weekly in the first 2 months after transplantation and then every 2 to 3 months until 3 years after the allograft.

Graft rejection was defined as either the absence of hematopoietic reconstitution of donor origin on day +45 after the allograft (primary graft rejection) or as loss of donor cells after a transient engraftment of donor-origin hematopoiesis, with return to transfusion dependence (secondary graft rejection).

aGvHD was graded according to the Seattle criteria.[18] Patients were considered to be evaluable for aGvHD if they survived for at least 7 days after bone marrow transplantation (BMT).

Overall survival (OS) was defined as the time interval between transplantation and death due to any cause, whereas thalassemia-free survival (TFS) was defined as the time interval from HSCT to first event (either death or complete, spontaneous autologous hematopoietic reconstitution or infusion of cryopreserved recipient hematopoietic stem cells).

Statistical analysis

Patient-, disease- and transplant-related variables were expressed as median and range or as percentage, as appropriate. The following patient or graft characteristics were analyzed for their potential impact on the outcome:

Table 2. HLA-DPB1 mismatches and classification as permissive or nonpermissive in UD-recipient pairs from this study

Classification* and UPN	Outcome†	HLA-DPB1‡ Recipient	HLA-DPB1‡ Donor
Permissive (n = 24)			
1	—	0201	0201,0401
2	—	0201,0402	0201,0401
3	—	0401	0101,0401
4	—	0401,1101	0401,0402
5	—	0401	0401,0402
6	R	0201,0401	0401
7	R	0401	0201,0401
8	III	*0301*,1701	0401,1701
9	—	0201,0402	0402,1101
10	—	0201,0401	0401
11	—	0201,0402	0201,0401
12	—	*0301*,0402	*0301*,0401
13	II	0201,*0301*	*0301*,0401
14	—	0401	0401,0402
15	II	0201	0401
16	—	0402	0202,0401
17	—	0401	0201
18	—	0201	0401,0402
19	—	*1401*	*0301*
20	—	0101	0101,0401
21	—	0402,1601	0201,0401
22	—	0401,0402	0201,0401
23	II	0402	0402,1101
24	II	*0301*,0401	0201,*0301*
Nonpermissive HvG (n = 17)			
25	II	0401,2401§	*0301*,0401
26	R	0201,0401	0201,*0301*
27	II	0401	0*0301*,0401
28	II	0201,0401	*0301*,0401
29	—	0401,0402	*0301*,0402
30	—	0201,0401	*0301*,0401
31	—	0401,0402	0401,1001
32	II	0201	*0301*,0402
33	IV	0201	*0301*,0401
34	R	0401,0402	0201,*0301*
35	R	0201,1501	0401,*1401*
36	II, R	0401	*0301*,1301
37	—	0401,0601	1001,2301
38	—	*0301*,0401	1001
39	—	0201	*0301*,0401
40	II	0201,0401	*0301*,1701
41	IV	0201	*0301*,0401
Nonpermissive GvH (n = 10)			
42	R	0401,1001	*0301*,0401
43	—	0201,*0301*	0201
44	III	0401,1001	0401,0501
45	—	*0301*,0401	0401,0402
46	—	1101,1701	0101,1101
47	—	*0301*,1701	0401,0402
48	III	1001	*0301*,1601
49	III	*0301*,0402	0401
50	—	*0301*,0401	0401
51	II	0401,1001	0402,2001

UPN indicates unique patient number; —, neither GVHD nor rejection.

*Classification of HLA-DPB1 mismatches as permissive or nonpermissive in HvG or GvH direction, according to the algorithm we previously described.[13]

†Outcome of each patient receiving a transplant in terms of occurrence of graft rejection (R) or acute GvHD grade II (II), III (III), or IV (IV), defined according to the criteria stated in "Patients and methods."

‡Complete typing of HLA-DPB1 alleles present in the recipient and in the donor. Immunogenicity groups of HLA-DPB1 alleles according to the algorithm we previously described[13] are indicated as follows: group 1, highly immunogenic (underlining); group 2, intermediately immunogenic (italics); group 3, poorly immunogenic (normal typeface).

§HLA-DPB1*2401 was assigned to group 3 of nonimmunogenic alleles on the basis of amino acid sequence homology with DPB1*4601, the prototype of group 3 alleles, and not, as all other alleles, on the basis of functional studies using alloreactive T lymphocytes.[13]

donor and recipient sex, donor and recipient age, patient Pesaro class at HSCT, HCMV serology, conditioning regimen, use of ATG, marrow cell dose infused, and type of HLA-DPB disparity.

For the analysis, continuous variables were categorized as follows: each variable was first divided into 4 categories at the 25th, 50th, and 75th percentiles. If the relative event rates (ratio of the observed number of events to the expected number of events in the category, assuming no variation across categories) in 2 or more adjacent categories (and the median times-to-events) were not different, these categories were grouped. If no clear pattern was observed for the primary outcomes, the median was taken as cut point.[19]

Patients were censored at time of rejection, death, or last follow-up. The probability of survival and TFS was estimated by the Kaplan-Meier method and expressed as percentage and 95% CI.[20] aGvHD occurrence and rejection probability were expressed as cumulative incidence curves, to adjust the analysis for competing risks.[21,22] The significance of differences between curves was estimated by the log-rank test. Furthermore, the differences in percentages of events in the subgroups of patients were also compared with the Fisher exact test or the χ^2 test, as appropriate. All variables with a *P* value below .5 in univariate analysis were included in a multivariate analysis performed using the Cox proportional hazard regression model.[23,24]

P less than .05 was considered statistically significant, *P* values from .05 to .5 were considered not statistically significant but were shown in the tables in detail; *P* of .5 or greater was reported as not significant (NS).

Statistical analysis was performed using the SAS System (SAS, Cary, NC) and the NCSS computer program (J. Hintze, 2001, NCSS and PASS, Number Cruncher Statistical System, Kaysville, UT).

Results

Classification of donor-recipient pairs according to an algorithm of HLA-DPB1 disparities

Seventy-two patients with β-thalassemia, given a transplant from a UD matched by high-resolution molecular typing for HLA-A, -B, -C, -DRB, and -DQB1, were studied. In 21 donor-recipient pairs (29%), both HLA-DPB1 alleles were matched (Table 1). The remaining pairs had one or 2 HLA-DPB1 disparities, which were classified as permissive (24 pairs, 33%) or nonpermissive in HvG (17 pairs, 24%) or GvH (10 pairs, 14%) direction, according to the algorithm we previously described.[13] Briefly, HLA-DPB1 alleles were classified into 3 groups with high (group 1: DPB1*0901; *1001; *1701), intermediate (group 2: DPB1*0301; *1401; *4501), or low (group 3: all other DPB1 alleles identified in these pairs) immunogenicity. An HLA-DPB1 mismatch was considered as permissive when the 2 mismatched alleles belonged to the same immunogenicity group. Instead, a nonpermissive HLA-DPB1 disparity in HvG direction was assigned when the donor's allele belonged to a higher immunogenicity group as compared to the patient's allele. Vice versa, an HLA-DPB1 mismatch was defined as nonpermissive in GvH direction when the patient's allele belonged to a higher immunogenicity group as compared to the donor's allele. A list of the HLA-DPB1 alleles in the 51 mismatched pairs, along with their classification according to our algorithm, is given in Table 2. In the permissive, the HvG and the GvH groups, a single HLA-DPB1 allele was mismatched in 18, 6, and 6 pairs, respectively, whereas both alleles were mismatched in 6, 11, and 4 pairs, respectively (Table 2).

Association of nonpermissive HLA-DPB1 disparities with graft rejection

Seven of 72 patients (10%) experienced graft rejection (Table 1). Four of these 7 patients had primary graft failure and 2 of them

required the reinfusion of autologous bone marrow harvested and cryopreserved before the allograft; the remaining 3 patients experienced secondary graft rejection and none of them developed cytopenia. Two of these latter 3 patients underwent successful retransplantation from the same donor. Only one (experiencing primary graft failure) of the patients who rejected the graft died, confirming that graft rejection is not invariably associated with mortality in thalassemia patients.

None of the 7 patients who had graft rejection was matched for both HLA-DPB1 alleles, and 2 patients belonged to the group with permissive DPB1 mismatches (Table 2). Overall, the cumulative incidence of rejection in the HLA-DPB1 matched or permissively mismatched group was 7% (95% CI, 2-26; Figure 1A). In contrast, the remaining 5 patients with rejection belonged to the group with nonpermissive HLA-DPB1 mismatches. The overall cumulative incidence of rejection in this group was 19% (95% CI, 9-43). Interestingly, in 4 of these cases, the mismatch was in HvG direction (Table 2), resulting in a significantly higher cumulative incidence of rejection in the HvG (26%; 95% CI, 11-60) as compared to the GvH group (10%; 95% CI, 2-64; Figure 1A). The comparison of the cumulative incidence of graft rejection between patients given transplants from donors with mismatch in HvG direction and patients receiving transplants from matched or permissively mismatched UDs is statistically significant ($P < .05$). In both univariate and multivariate analysis, only the presence of disparity in HvG direction was statistically associated with the occurrence of graft rejection (Tables 3 and 6 provide further details). No other factor influenced the risk of graft rejection.

Association of nonpermissive HLA-DPB1 disparities with aGvHD grade II-IV

One of the 72 patients studied, belonging to the nonpermissive groups, was not informative for aGvHD, due to early death. Overall, 26 of 71 evaluable patients (37%) developed grade II-IV aGvHD (Tables 1 and 4). Fourteen of these patients belonged to the HLA-DPB1 allele matched or permissively mismatched group, resulting in an overall cumulative incidence of grade II-IV aGvHD of 31% (95% CI, 20-49; Figure 1B) in these 2 groups taken together. The remaining 12 patients who developed grade II-IV aGVHD belonged to the nonpermissive groups (Table 2). The cumulative incidence of grade II-IV aGvHD in the nonpermissive groups was slightly higher (46%; 95%CI = 24-65) as compared to patients belonging to the HLA-DPB1 allele-matched or permissively mismatched group, but this difference was not statistically significant (Figure 1B; Table 4). In concordance with our previous observations obtained in patients undergoing transplantation for hematologic malignancies,[13] the incidence of grade II-IV aGvHD was equally distributed between patients given the allograft from a donor with nonpermissive mismatches in either GvH or HvG direction (Figure 1B and Table 4 present further details). No other variable influenced the probability of developing grade II-IV acute GVHD (Table 4).

Association of nonpermissive HLA-DPB1 mismatches with TFS and OS

TFS was reduced in patients with nonpermissive HLA-DPB1 mismatches in HvG (10 of 17; 59%) or GvH (6 of 10; 60%) direction, as compared to the matched or permissive group (35 of 45; 78%; Table 1). The difference in terms of Kaplan-Meier estimates of TFS for the 3 subgroups was, however, not statistically significant (Figure 1C; Table 5). No statistically significant association was observed between nonpermissive HLA-DPB1 mismatches in either HvG or GvH direction and the rate of OS (Tables 1 and 5). In multivariate analysis, HLA-DP disparity in HvG direction was associated with a statistically significant, lower probability of TFS as compared to patients belonging to the matched or permissive group (RR = 5.15; 95% CI = 1.58-16.82; $P = .01$). Donor age older than 35 years was also associated with a statistically significant, lower probability of being alive and transfusion independent (RR = 3.21; 95% CI = 1.05-9.83; $P = .01$). No other variable statistically influenced the probability of TFS, although there was a trend for a greater risk of treatment failure in men as compared to women (Table 6).

Discussion

Although allogeneic HSCT is the only definitive cure for β-thalassemia, the nonmalignant nature of this disease and the continuous improvement obtained with conservative treatment calls for careful evaluation of potential risks and benefits of this therapeutic option. The increased incidence of graft rejection observed when UDs are used is an argument that may discourage the application of UD HSCT to patients with thalassemia.

The results of this study indicate that the risk of rejection might be reduced to low levels, comparable with those of HLA-identical sibling transplantation,[8,9] by selecting UDs according to stringent rules of immunogenetics. In particular, for reaching this objective, UDs matched at the allelic level for HLA-A, -B, -C, -DRB, and -DQB1 loci should not present nonpermissive mismatches at HLA-DPB1 in the HvG direction. We used the definition of nonpermissive HLA-DPB1 disparities using an algorithm that we

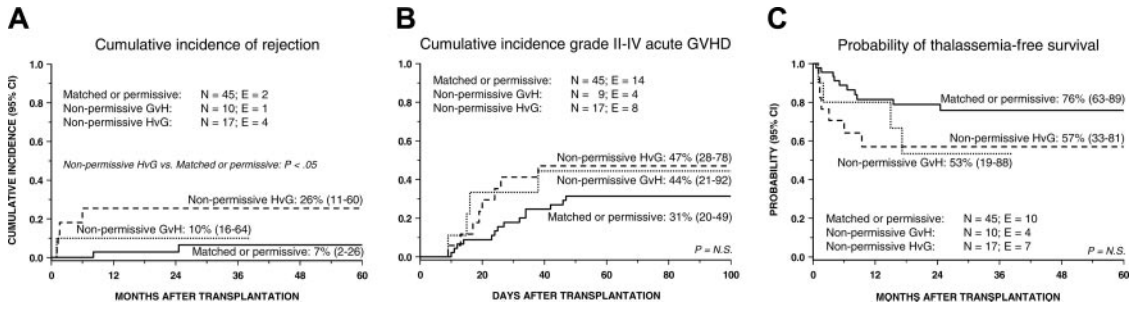

Figure 1. Influence of HLA-DPB1 mismatches on the outcome of UD-SCT for β-thalassemia. The probability of graft rejection (A), grade II-IV aGvHD (B), and TFS (C) was analyzed by cumulative incidence (A-B) or Kaplan-Meier estimates (C) in 72 (A,C) or 71 (B) evaluable patients. Patients and donors were classified as: (1) matched for HLA-DPB1 or with a permissive mismatch (solid line; n = 45); (2) matched for HLA-DPB1 with nonpermissive DPB1 mismatches in HvG direction (broken line; n = 17); (3) matched for HLA-DPB1 with nonpermissive DPB1 mismatches in GvH direction (dotted line; n = 10 [A,C] or n = 9 [B]), according to the algorithm we previously described.[13]

Table 3. Univariate analysis for the risk of graft rejection

Variable	No. patients	No. events (%)	Fisher exact test *P*	Cumulative incidence of rejection, % (95% CI)	*P*
Sex			.46		.43
Female	31	2 (6)		8 (2-29)	
Male	41	5 (12)		18 (6-35)	
Sex mismatch in GvH direction, donor→recipient			NS		NS
Female→male	19	2 (11)		13 (3-47)	
Other combinations	53	5 (9)		12 (5-27)	
Sex mismatch in HvG direction, donor→recipient			NS		NS
Male→female	18	1 (6)		8 (1-51)	
Other combinations	54	6 (11)		13 (3-47)	
Patient age at HSCT			NS		NS
Younger than 15 y	36	4 (11)		14 (5-35)	
15 y or older	36	3 (8)		9 (3-26)	
Donor age			.46		.45
Younger than 35 y	31	2 (6)		7 (2-25)	
35 y or older	41	5 (12)		15 (7-35)	
Pesaro classification			NS*		NS
Class 1	21	3 (14)		18 (6-51)	
Class 2	20	1 (5)		10 (3-37)	
Class 3	31	2 (6)		7 (2-25)	
HCMV serology, donor→recipient			.50*		NS
Negative→negative	7	1 (14)		17 (3-100)	
Negative→positive	16	3 (19)		24 (9-65)	
Positive→negative	14	1 (7)		7 (1-47)	
Positive→positive	35	2 (6)		6 (2-23)	
HCMV serology combination, donor→recipient			NS		NS
Negative→negative	7	1 (14)		17 (3-100)	
Others	65	6 (9)		11 (5-24)	
Conditioning regimen			.14*		.14
BU + CY	18	3 (17)		17 (6-48)	
BU + TT + CY	36	1 (3)		3 (0-20)	
BU + TT + FLU	18	3 (17)		23 (8-63)	
Use of ATG			.36		.32
No	54	4 (7)		8 (3-19)	
Yes	18	3 (17)		23 (8-63)	
Cell dose infused, × 10^8/kg			NS		NS
Less than 5	44	4 (9)		9 (4-24)	
5 or greater	28	3 (11)		14 (5-40)	
HLA-DPB1 mismatch:			.08*		.048
Matched + permissive	45	2 (4)		7 (2-26)	
Nonpermissive mismatch in GvH direction	10	1 (10)		10 (2-64)	
Nonpermissive mismatch in HvG direction	17	4 (24)		26 (11-60)	

*Chi-squared *P*.

previously defined. This algorithm, developed through an analysis of patients with heterogeneous hematologic malignancies given disparate GvHD prophylaxis and conditioning regimens,[13] is based on functional evidence from alloreactive T cells and on the principles of central T-cell tolerance.[14] Because the alloreactive T cells studied were derived from a patient expressing self–HLA-DPB1*0201,[25] a potential immunogenicity of DPB1*0201 might have been undetected, as self-reactive T cells are likely to have been deleted in this patient. However, the presence of HLA-DPB1*0901-specific T cells in this same patient[25] demonstrates that any immunogenic epitope of DPB1*0201 should be distinct from the epitope shared between DPB1*0901 and the other immunogenic alleles considered in our algorithm. Moreover, when HLA-DPB1*0201 was presented together with DPB1*1001 on the same antigen-presenting cell to HLA-A, -B, -C, -DRB, -DQB1–matched responder cells expressing DPB1*0101,*0402 of group 3, the vast majority of HLA-DP–specific T-cell clones was found to be directed against DPB1*1001, this finding suggesting a stronger immunogenicity mediated by this allele in vitro (K.F., personal unpublished data, October 2004). From a practical viewpoint, the information obtained in this study implies that in donor-recipient pairs of white origin, about 25% of recipients may be exposed to an increased incidence of graft rejection due to a nonpermissive HLA-DPB1 mismatch in HvG direction,[13] whereas for the remaining patients the risk of lack of sustained engraftment of donor cells can be estimated to be similar to that of patients who have an HLA-identical sibling donor available. Importantly, this finding was not influenced by the disease risk classification (class 1, 2, or 3),[9] which has been shown to have an important impact on the outcome of allogeneic HSCT for β-thalassemia, and, in particular, which was reported to be associated with the risk of rejection of HLA-identical sibling transplants.[8,9] It is interesting to note that targeted avoidance of nonpermissive HLA-DPB1 mismatches in HvG direction allows clinicians to offer transplantation to more than 75% of patients with an HLA-A, -B, -C, -DRB, -DQB1 matched donor, whereas

Table 4. Univariate analysis for the risk of grade II–IV a GVHD

Variable	No. patients	No. events (%)	Fisher exact test *P*	Cumulative incidence of GvHD, % (95% CI)	*P*
Sex			NS		NS
Female	31	12 (39)		39 (25-61)	
Male	40	14 (35)		35 (23-53)	
Sex mismatch in GvH direction, donor→recipient			NS		NS
Female→male	18	6 (33)		33 (17-64)	
Other combinations	53	20 (38)		38 (27-54)	
Sex mismatch in HvG direction, donor→recipient			.30		.50
Male→female	18	8 (44)		46 (27-77)	
Other combinations	53	18 (34)		34 (23-49)	
Patient age at HSCT			NS		NS
Younger than 15 y	35	13 (37)		37 (24-57)	
15 y or older	36	13 (36)		37 (24-57)	
Donor age			.32		.33
Younger than 35 y	31	9 (29)		29 (17-51)	
35 y or older	40	17 (43)		43 (30-61)	
Pesaro classification			NS*		NS
Class 1	21	7 (33)		33 (18-61)	
Class 2	19	6 (32)		32 (16-61)	
Class 3	31	13 (42)		43 (28-64)	
HCMV serology, donor→recipient			.32*		.33
Negative→negative	7	3 (43)		43 (18-100)	
Negative→positive	15	3 (20)		20 (7-55)	
Positive→negative	14	4 (29)		29 (12-65)	
Positive→positive	35	16 (46)		46 (32-66)	
HCMV serology combination, donor→recipient			NS		NS
Negative→negative	7	3 (43)		43 (18-100)	
Others	64	23 (36)		36 (36-50)	
Conditioning regimen			NS*		NS
BU + CY	17	6 (35)		35 (19-67)	
BU + TT + CY	36	13 (36)		36 (24-56)	
BU + TT + FLU	18	7 (39)		39 (22-69)	
Use of ATG			NS		NS
No	53	19 (36)		36 (25-52)	
Yes	18	7 (39)		39 (22-69)	
Cell dose infused, × 10^8/kg			.21		.19
Less than 5	43	13 (30)		31 (19-48)	
5 or greater	28	13 (46)		46 (31-69)	
HLA-DPB1 mismatch			.44*		.31
Matched + permissive	45	14 (31)		31 (20-49)	
Nonpermissive mismatch in GvH direction	9	4 (44)		44 (21-92)	
Nonpermissive mismatch in HvG direction	17	8 (47)		47 (28-78)	

*Chi-squared *P*.

this number drops to 30% if only fully DPB1 matched pairs were selected for transplantation.

Another intriguing observation is that the impact of nonpermissive HLA-DPB1 mismatches on rejection is similar to that correlated to the presence of class I disparities at the allelic level in pairs matched or permissively mismatched at the HLA-DPB1 locus. In fact, the cumulative incidence of graft rejection of our patients with a nonpermissive HLA-DPB1 mismatch in HvG direction is 26%, a value comparable to that of 33% that we observed in 15 different patients, not included in this analysis because they had been given transplants from an HLA class I disparate UD. This observation further supports the functional importance of nonpermissive HLA-DPB1 mismatches, which was also immunologically demonstrated in a study documenting that an epitope encoded by HLA-DPB1 can be the target of cytotoxic $CD4^+$ T lymphocytes involved in allograft rejection.[25]

UD HSCT for β-thalassemia is an ideal model to study the biologic role of defined immunogenetic factors, because we could investigate a cohort homogenous for disease, stem cell source used, and GvHD prophylaxis, and because such patients have an essentially fully functional immune system that has not been compromised by the underlying disease or by previous chemotherapy or immunosuppressive treatment. The significant association between the presence of nonpermissive HLA-DPB1 disparities and the occurrence of graft rejection, observed in the present study enrolling the largest group of UD HSCT available for this disease, underscores the biologic relevance of nonpermissive DPB1 mismatches. Noteworthy, this association was observed predominantly in the group with nonpermissive mismatches in the HvG direction, consistent with the notion that graft rejection could have been mediated by alloreactive recipient T cells recognizing immunogenic HLA-DP alloantigens on donor cells. This effect could be either direct or indirect, by providing help to minor histocompatibility antigen-specific cytotoxic effector T cells, or to B cells producing HLA-DP–specific alloantibodies. Such alloantibodies might also be directly reactive with the shared immunogenic T-cell epitope of group 1 and 2 alleles. In agreement with this hypothesis, it has recently been shown that virtually all HLA-DP–specific

Table 5. Univariate analysis for the probability of TFS

Variable	No. patients	No. events (%)	Fisher exact test *P*	Kaplan-Meier probability (95% CI)	*P*
Sex			.13		.14
Female	31	6 (19)		79 (64-94)	
Male	41	16 (39)		61 (46-77)	
Sex mismatch in GvH direction, donor→recipient			.40		.36
Female→Male	19	7 (37)		62 (39-84)	
Other combinations	53	14 (26)		71 (58-84)	
Sex mismatch in HvG direction, donor→recipient			NS		NS
Male→female	18	5 (28)		69 (47-92)	
Other combinations	54	16 (30)		69 (56-81)	
Patient age at HSCT			.45		.39
Younger than 15 y	36	9 (25)		73 (57-88)	
15 y or older	36	12 (33)		65 (49-81)	
Donor age			.31		.40
Younger than 35 y	31	7 (23)		77 (62-92)	
35 y or older	41	14 (34)		63 (47-79)	
Pesaro classification			.12*		.16
Class 1	21	4 (19)		77 (56-97)	
Class 2	20	4 (20)		80 (62-98)	
Class 3	31	13 (42)		57 (39-75)	
HCMV serology, donor→recipient			.23*		.31
Negative→negative	7	2 (29)		71 (38-100)	
Negative→positive	16	6 (38)		59 (34-85)	
Positive→negative	14	1 (7)		93 (79-100)	
Positive→positive	35	12 (34)		64 (47-80)	
HCMV serology combination, donor→recipient			NS		NS
Negative→negative	7	2 (29)		71 (38-100)	
Others	65	19 (29)		68 (56-80)	
Conditioning regimen			NS*		NS
BU + CY	18	6 (33)	66 (44-88)		
BU + TT + CY	36	11 (31)		69 (53-84)	
BU + TT + FLU	18	4 (22)		70 (45-95)	
Use of ATG			NS		.45
No	54	17 (31)		68 (55-80)	
Yes	18	4 (22)		70 (45-95)	
Cell dose infused, $\times 10^8$/kg			.30		.18
Less than 5	44	15 (34)		64 (50-79)	
5 or greater	28	6 (21)		75 (58-93)	
HLA-DPB1 mismatch			.25*		.18
Matched + permissive	45	10 (22)		76 (63-89)	
Nonpermissive mismatch in GvH direction	10	4 (40)		53 (19-88)	
Nonpermissive mismatch in HvG direction	17	7 (41)		57 (33-81)	

*Chi-squared *P*.

alloantibodies from sensitized kidney retransplant patients recognized an epitope encoded by DPB1*0301, which belongs to immunogenic group 2 of our algorithm.[26] The presence of recipient-derived HLA-specific alloantibodies and cytotoxic T-lymphocytes directed against minor histocompatibility antigens may be also involved in rejection occurring in thalassemia patients receiving transplants from HLA-identical siblings. Patient spleen enlargement and ineffective exposure to the myeloablative agent BU, due to interpatient pharmacokinetics variability,[27] may contribute to rejection as well.

The results from this study, together with those obtained from the analysis previously performed on patients with hematologic malignancies,[13] confirm that matching for HLA-DPB1 might not require complete typing for all 119 alleles known to date, but might be limited to identifying the presence or absence of the immunogenic sequences by a restricted number of allele-specific amplifications or probe hybridizations, thus sparing costs and time.

The failure, in the past, to document a significant clinical impact of HLA-DPB1 disparities on the outcome of patients undergoing allogeneic UD HSCT could have been partly due to the obscuring effect of molecular mismatches at HLA class I loci, undetected when serologic class I typing is used.[28,29] As all our donor-recipient pairs were matched at the allelic level for HLA-A, -B, -C, -DRB, and -DQB1 loci, we can speculate, in accordance with previous reports,[12,30] that this condition is ideal to dissect any impact of HLA-DP disparities on posttransplantation outcomes. The extremely low incidence of rejection (1 of 118), favored by impairment of immune system due to chemotherapy, in the cohort of patients with hematologic malignancies analyzed in our previous report,[13] precluded the possibility to detect any association of nonpermissive HLA-DPB1 mismatches with graft rejection.

Although we found a significant correlation between nonpermissive HLA-DPB1 mismatches and occurrence of grade II-IV aGvHD in patients with malignancies,[13] this correlation was less

Table 6. Multivariate analysis on the impact of HLA-DPB1 mismatches on transplantation outcome in patients with thalassemia

Variable	Relative risk (95% CI)	*P*
Risk of rejection		
Patient sex		
Male vs. female	1.64 (0.29-9.34)	NS
Donor age		
35 y or older vs younger than 35 y	2.57 (0.45-14.59)	.29
Conditioning regimen		
BU + TT + CY vs BU + CY	0.12 (0.01-1.25)	.08
BU + TT + FLU vs BU + CY	0.88 (0.23-1.90)	NS
Use of ATG		
Yes vs no	0.77 (0.15-4.10)	NS
HLA-DPB1 mismatch		
Nonpermissive mismatch in GvH direction vs matched + permissive	3.98 (0.35-45.24)	.27
Nonpermissive mismatch in HvG direction vs matched + permissive	7.42 (1.29-42.68)	.02
Grade II–IV aGvHD*		
Sex mismatch in HvG direction		
Male donor and female recipient vs others	1.48 (0.62-3.52)	.37
Donor age		
35 y or older vs younger than 35 y	2.13 (0.91-4.98)	.08
HCMV serology, donor→recipient		
Negative→positive vs negative→negative	0.49 (0.09-2.75)	.42
Positive→negative vs negative→negative	0.85 (0.18-4.06)	NS
Positive→positive vs negative→negative	1.63 (0.42-6.31)	.48
Cell dose infused, $\times 10^8$/kg		
5 or more vs less than 5	2.40 (0.94-5.55)	.07
HLA-DPB1 mismatch		
Nonpermissive mismatch in GvH direction vs matched + permissive	2.87 (0.83-9.94)	.10
Nonpermissive mismatch in HvG direction vs matched + permissive	2.21 (0.85-5.78)	.11
TFS probability		
Patient sex		
Male vs female	3.16 (0.96-10.40)	.06
Sex mismatch in GvH direction		
Female donor and male recipient vs others	1.08 (0.28-4.14)	NS
Patient age at HSCT		
15 y or older or younger than 15 y	0.65 (0.15-2.81)	NS
Donor age		
35 y or older vs younger than 35 y	3.21 (1.05-9.83)	.04
Pesaro class		
Class 1	—	—
Class 2	0.79 (0.13-4.64)	NS
Class 3	3.17 (0.41-24.65)	.27
HCMV serology, donor→recipient		
Negative→positive vs negative→negative	3.89 (0.54-28.30)	.18
Positive→negative vs negative→negative	0.29 (0.02-3.84)	.35
Positive→positive vs negative→negative	2.54 (0.40-16.24)	.32
Use of ATG		
Yes vs no	1.97 (0.44-8.79)	.38
Cell dose infused, $\times 10^8$/kg		
5 or greater vs less than 5	0.39 (0.11-1.34)	.13
HLA-DPB1 mismatch		
Nonpermissive mismatch in GvH direction vs matched + permissive	1.48 (0.34-6.51)	NS
Nonpermissive mismatch in HvG direction vs matched + permissive	5.15 (1.58-16.82)	.01

Results of multivariate analysis including relative risk (RR) for rejection, grade II–IV aGvHD and mortality, 95% CI, and *P* values associated with HLA-DPB1 disparities.
— indicates the reference groups.
*71 of 72 pairs were evaluable for aGvHD (45 matched + permissive, 9 nonpermissive in GvH and 17 nonpermissive in HvG direction).

pronounced and not statistically significant in the present study. This could be due to differences in strategy for GvHD prophylaxis and in conditioning regimens, or to different immunologic conditions in patients who are not pretreated by chemotherapy. As in the previous report, the increased risk for aGvHD was associated with nonpermissive HLA-DPB1 mismatches in either GvH or HvG direction. One possible explanation for this observation may rely on the complexity of the pathophysiologic mechanisms underlying aGvHD, which include, in addition to a direct effect mediated by host-specific donor-derived T cells, indirect effects mediated by inflammatory cytokines.[31,32] It is possible that the immune response induced by nonpermissive mismatches in HvG direction might lead to the release of cytokines that, in turn, facilitate the onset of aGvHD in these patients. In the leukemia cohort, nonpermissive HLA-DPB1 mismatches were also associated with a marked though not statistically significant reduction of OS, especially in the HvG group, due to a higher incidence of relapse.[13] In the present study, in multivariate

analysis, we found a statistically significant greater risk of treatment failure in the presence of nonpermissive HLA-DPB1 mismatches as compared to the HLA-DP matched or permissive mismatched group. The detrimental effect played by an advanced donor age on posttransplantation outcome found in our study is in agreement with a previously published analysis on around 7000 allografts from unrelated volunteers facilitated by the National Marrow Donor Program, which showed that the use of younger donors improved survival.[33]

Overall, the data from this study offer useful rules, based on immunogenetic criteria, for the selection of the "best" UD for patients with β-thalassemia, avoiding the selection of donors with nonpermissive HLA-DP disparity. Moreover, knowledge of the innocuousness of mismatches classified as permissive could permit selection with more confidence of donors mismatched at the HLA-DP locus without further extenuating searches for a fully matched donor.

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