Bone Marrow Research

Biological and Genetic Aspects of Donor-Recipient Matching in HSCT

Guest Editors: Andrzej Lange, Colette Raffoux, and Bronwen Shawx

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Editorial Board

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 212593, 2 pages
doi:10.1155/2012/212593

Editorial

Biological and Genetic Aspects of Donor-Recipient Matching in HSCT

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Received 20 November 2012; Accepted 20 November 2012

Bone marrow transplantation is a routine clinical activity offering salvage therapy in a number of hematological diseases and inborn errors. There are two obstacles that may delay or even postpone this curable treatment approach. The first is a lack of matched family donors, which affects up to 75% of patients. In this situation a search for unrelated donors, if successfully completed, makes this approach feasible. HLA genes of five loci (A, B, C, DR, and DQ) are currently considered as a basis for matching. Each day brings information of new alleles. Genetic typing can lead to detection of diversity at the single nucleotide level. It ensures that a level of matching is achieved resulting in transplant success rates similar to those seen among siblings sharing the same HLA genotype. While we wish to have a perfect match, also important is elapsing time during the search process, which is related to the presence in the patient of rare alleles and unusual B-C, DR-DQ associations. Having a primary typing of a patient we can predict the chance for a proper match. Each day new donors are recruited worldwide. Iterative searching must be applied in the latter situation. In some cases 6 or more potential donors are required to have a donor accepted by a clinician. Finally, a compromise must be reached between the aspiration of matching at the level of 10 alleles and the urgency of transplantation in patients suffering from relapsing disease. To facilitate the decision-making process, modern information technology must be at hand. The search process includes the complete donor pool which is screened for potential donors. The chosen potential donors must be activated for confirmatory typing which includes 5 loci specificities typed at the high resolution level with exchange of information between registries and the hospital iteratively coming to the optimal decision. The process must be reliable, safe, and transparent, and must operate efficiently in real time. The European Marrow Donor Information System (EMDIS), used in many countries worldwide, ensures fulfillment of the above requirements. The present volume illustrates the above points, supporting the rational basis for the decision-making process.

Identification of HLA alleles in populations with a genetic background composed of different ancestral gene compositions may depict the prevalent component in the ethnicity. The latter can not only facilitate the search process but also provide some information on the presence of factors modifying the risk of graft-versus-host disease. You can read about that in this volume. Non-HLA genetic factors influencing the natural history of hematological malignancies and also shaping the risk of post-HSCT complications are being investigated by several groups of investigators. Among non-HLA genetic factors, probably killer immunoglobulin-like receptors (KIRs) and factors associated with the NOD2/CARD15 polymorphism have the best reputation for influencing the outcome of HSCT. Described and then consequently typed in alloHSCT patients KIR haplotypes associate with the ability of an individual to mount an immune response. Single-nucleotide polymorphism of the *NOD2* gene influences inflammatory response to the bacterial cell wall components that may induce advert effects. Both associations discussed in this volume document the significance of environmental factors including infections for the overt clinical manifestation of a primary alloreactive response. Indeed,

in another paper in this volume, the associations between bacterial infections, *NOD2* gene mutation associated features, and the vigorous immune response involving a proinflammatory T cell subpopulation producing IL-17 in mounting graft-versus-host disease are shown and illustrated. The role of non-genetic factors, useful during the donor-recipient matching process, is described using the example of seropositivity against CMV in donors and recipients. The absence of CMV IgG antibodies in donors constitutes a risk factor of reactivation of this virus and influences the incidence of aGvHD of patients post HSCT. Therefore, biological factors modifying the outcome of HSCT may include HLA and non-HLA genetic variant associated features.

Genetic diversity and non-genetic factors influence the outcome of HSCT, which renders the process of matching a very complex task. To facilitate the final decision it is important to recognize the presence of different factors and then to put them in an order depending on their weights. The present special issue of Bone Marrow Research presenting genetic and non-genetic factors affecting the outcome of HSCT may serve as a complementary tool in the decision-making process while choosing an optimal donor for a given patient. Each pair should be analyzed for the presence or absence of genetic traits or non-genetic characteristics which in a complementary fashion may influence the outcome of HSCT. We hope that the present volume contributes some additional information supporting this notion.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 375075, 8 pages
doi:10.1155/2012/375075

Clinical Study

CMV Serostatus of Donor-Recipient Pairs Influences the Risk of CMV Infection/Reactivation in HSCT Patients

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Received 28 July 2012; Revised 24 September 2012; Accepted 24 September 2012

Academic Editor: Bronwen Shaw

CMV donor/recipient serostatus was analyzed in 200 patients allografted in our institution from unrelated (122 patients) donors and 78 sibling donors in the years 2002–2011 in relation to posttransplant complications. On a group basis independently of the CMV serostatus of donor-recipient pairs sibling transplantations and those from unrelated donors that matched 10/10 at allele level had a similar rate of CMV reactivation (17/78 versus 19/71, P = ns). The rate of CMV reactivation/infection was higher in patients grafted from donors accepted at the lower level of matching than 10/10 (18/38 versus 36/149, $P = 0.008$). The incidence of aGvHD followed frequencies of CMV reactivation in the tested groups, being 40/156 and 25/44 in patients grafted from sibling or unrelated donors that 10/10 matched and in those grafted from donors taht HLA mismatched, respectively ($P = 0.001$). Regarding the rate of reactivation in both groups seropositive patients receiving a transplant from seronegative donors had more frequently CMV reactivation as compared to those with another donor-recipient matching CMV serostatus constellation (22/43 versus 32/143, $P = 0 < 0.001$). Multivariate analysis revealed that seropositivity of recipients with concomitant seronegativity of donors plays an independent role in the CMV reactivation/infection (OR = 2.669, $P = 0.037$; OR = 5.322, $P = 0.078$; OR = 23.034, $P = 0.023$ for optimally matched and mismatched patients and the whole group of patients, resp.).

1. Introduction

Donor-recipient matching for unrelated hematopoietic stem cell transplantation (HSCT) in addition to human leukocyte antigens (HLA) includes CMV serostatus of the donor and recipient to facilitate the decision [1, 2].

In the clinical practice the presence of CMV IgM antibodies is suggestive of the active infection/reactivation and the presence of IgG antibodies indicates prior infection and shows CMV immunological competence of individuals [3–5]. Unfortunately, it is very suggestive that IgG CMV antibody positive individuals harbor CMV in a latent form and their blood products are infective for CMV incompetent recipients [6]. In the present era of specific anti-CMV chemotherapy the significant impact of pretransplant donor seropositivity on the patient outcome is controversial—reviewed in the Boeckh and Nichols paper [7]. However, recipient CMV serostatus still remains an important risk factor of the patient outcome [8, 9].

HSCT involving pairs in which both donor and recipient lack CMV IgG antibodies is associated with a lower transplant mortality [10]. In the latter situation we are dealing with a donor-recipient pair in which probably neither donor nor recipient has CMV in a latent form. On the other hand, positivity of both donor and recipient should also favor the HSCT outcome—both donors and recipients likely have CMV in a latent form but the immune system of the donor should have a memory of CMV infection, which facilitates the immune response to CMV posttransplant. However, Ljungman et al. [11] in the megafile analysis

Table 1: Patient characteristics.

Number of patients	200
Age	
(median, range), yrs	34, 1–60
Adults > 16 yrs	174
Children ≤ 16 yrs	26
Recipient gender	
Female	91
Male	109
Donor gender	
Female	83
Male	116
Donor	
Sibling	78
Unrelated HLA matched (10/10 at the allele level),	78
Mismatched, at the allele or low resolution levels up to two mismatches	44
Transplant material	
Bone marrow (BM)	28
Peripheral blood progenitor cells (PBPC)	172
Diagnosis	
Hematological malignancies (HM)	175
Chronic myeloid leukemia (CML)	24
Chronic lymphocytic leukemia (CLL)	5
Acute myeloid leukemia (AML)	67
Acute lymphocytic leukemia (ALL)	39
Other HM	40
Anemias and immunodeficiencies	24
Osteopetrosis	1
Conditioning regimen	
Myeloablative	105
Reduced intensity conditioning (RIC)	95
Acute GvHD, grades	
0	114
I	21
II	26
III	16
IV	23
Chronic GvHD	
Extensive	38
Limited	33
EBV ≥ 100 DNA copies/10^5 cells	45/187
CMV ≥ 100 DNA copies/10^5 cells	54/187
HHV6 ≥ 100 DNA copies/10^5 cells	34/187
Polyoma (JC/BK)	19/33
CMV IgG serostatus	
Recipients	
CMV IgG negative	32
CMV IgG positive	168
Donors	
CMV IgG negative	66
CMV IgG positive	132
Recipient/donor CMV serostatus	
Recipient CMV IgG (+)/donor CMV IgG (+)	118
Recipient CMV IgG (−)/donor CMV IgG (−)	18
Recipient CMV IgG (+)/donor CMV IgG (−)	48
Recipient CMV IgG (−)/donor CMV IgG (+)	14

showed that the latter important observation seems to be valid only for the unrelated donor transplantation setting.

To add new information to the still disputable association between the CMV donor/recipient serostatus with the outcome of transplantation the present study was undertaken.

2. Materials and Methods

Two hundred patients (F/M: 91/109; 26 and 174 patients were below and above 16 years of age, resp.) allografted from unrelated donors (122 patients), and 78 from sibling (SIB) donors in our institution in the years 2002–2011 were studied. One hundred and seventy-five suffered from hematological malignancies acute myeloid leukemia (AML; $n = 67$), chronic myeloid leukemia (CML; $n = 24$), acute lymphocytic leukemia (ALL; $n = 39$), other lymphoproliferative disorders ($n = 23$), myeloproliferative diseases ($n = 10$), and myelodysplastic syndromes ($n = 12$). The others were transplanted because of anemias (10 patients) and immunodeficiencies (14 patients) and osteopetrosis ($n = 1$).

One hundred and five and 95 patients received myeloablative (based on busulfan and cyclophosphamide) and reduced intensity conditioning (reduced busulfan dose or melphalan plus fludarabine and antithymocyte globulin (ATG)), respectively. Unrelated donor transplanted patients and those on reduced intensity conditioning received ATG (10 to 12.5 mg/kg b.w. cumulative dose, 125 patients) or alemtuzumab (90 mg as a dose, 38 patients) as a part of the conditioning regimen. All patients were on cyclosporin A with a dose adjusted to the blood CsA trough a level to 200 ng/L. CMV serostatus, age, gender, underlying disease, donor source, and HLA match as well as conditioning regimen (reduced or myeloablative) are given in Table 1.

The patients were routinely followed for clinical outcome in one-week intervals until 30 days posttransplant, then monthly until one year post-transplant and as well as when clinical symptoms were suggestive of CMV, EBV, or HHV6 reactivation or any other serious post-transplant complications including relapse or GvHD. Out of 200 patients studied viral CMV, EBV and HHV6 DNA copies in blood were determined in 187 recipients transplanted after the year 2003.

The Zeus Scientific, Inc. (NJ, USA), IgG and IgM ELISA test system was used for qualitative detection of CMV-specific antibodies in donors' and recipients' plasma. The ELISA kit was used according to the manufacturer's instructions. Briefly, microtiter plates, precoated with inactivated

Table 2: Univariate analysis of risk factors for aGvHD and CMV reactivation/infection event(s) in patients post-alloHSCT.

Variable	aGvHD		P value	CMV absence	CMV presence	P value
	≤grade I	>grade I		Infection/reactivation until 1 year post-HSCT		
Donor/recipient HLA match						
Matched	116	40	**$P < 0.001$**	113	36	**$P = 0.008$**
Mismatched at the allele or low resolution levels up to two mismatches	**19**	**25**		**20**	**18**	
Source of HSCT						
PBPC	**111**	**61**	**$P = 0.029$**	112	48	$P = 0.496$
BM	24	4		21	6	
Type of donor						
SIB	65	13	**$P < 0.001$**	61	17	**$P = 0.074$**
MUD	**70**	**52**		72	37	
Conditioning regimen						
RIC	67	28	$P = 0.450$	60	30	$P = 0.202$
Myeloablative	68	37		73	24	
Donor CMV IgG						
CMV IgG−	**38**	**28**	**$P = 0.036$**	**36**	**23**	**$P = 0.055$**
CMV IgG+	96	36		96	31	
Recipient CMV IgG						
CMV IgG−	19	13	$P = 0.307$	24	4	**$P = 0.073$**
CMV IgG+	116	52		**109**	**50**	
Donor-recipient IgG CMV serology						
R−/D−	10	8		15	1	
R+/D−	28	20	$P = 0.159$	**21**	**22**	**$P < 0.001$**
R−/D+	9	5		9	3	
R+/D+	87	31		87	28	
R−/D−, R+/D−, R−/D+ , R+/D+	106	44	$P = 0.115$	111	32	**$P < 0.001$**
R+/D−	28	20		**21**	**22**	
Donor/recipient gender						
Male to male, female to female, and male to female	105	54	$P = 0.572$	105	44	$P = 0.841$
Female to male	29	11		28	10	
Donor gender						
Male	76	40	$P = 0.543$	75	32	$P = 0.747$
Female	58	25		58	22	
Recipient gender						
Male	76	33	$P = 0.544$	71	27	$P = 0.747$
Female	59	32		62	27	
Recipient age						
≤16	17	9	$P = 0.824$	20	2	**$P = 0.042$**
>16	118	56		**113**	**52**	
CMV infection/reactivation event within 1 year post-HSCT						
CMV−	97	36	**$P = 0.025$**			
CMV+	**30**	**24**				

Table 2: Continued.

Variable	aGvHD		*P* value	CMV absence	CMV presence	*P* value
	≤grade I	>grade I		Infection/reactivation until 1 year post-HSCT		
aGvHD						
aGvHD ≤ grade I				97	30	***P* = 0.025**
aGvHD > grade I				**36**	**24**	
EBV infection/reactivation event within 1 year post HSCT						
EBV−	100	42	$P = 0.204$	104	38	$P = 0.263$
EBV+	27	18		29	16	
HHV6 infection/reactivation event within 1 year post HSCT						
HHV6−	103	50	$P = 0.835$	110	43	$P = 0.677$
HHV6+	24	10		23	11	

PBPC: peripheral blood progenitor cells; BM: bone marrow; R: recipient; D: donor; "−": negative; "+": positive; ATG: antithymocyte globulin; SIB: HLA-identical siblings; MUD: unrelated donors; RIC: reduced intensity conditioning.

Table 3: Multivariate analysis of risk factors for aGvHD (grade > I).

Variable	Coefficient	*P* value	Odds ratio	95% CI
CMV infection/reactivation event within 1 year post HSCT	0.5473	0.1362	1.7286	0.8415 to 3.5509
CMV IgG in donor serum	0.0290	0.9411	1.0295	0.4762 to 2.2254
Donor-recipient HLA mismatch	**0.8591**	**0.0421**	**2.3611**	**1.0310 to 5.4072**
Unrelated donor	**0.9520**	**0.0355**	**2.5909**	**1.0669 to 6.2921**
BM as a source of cells	−0.6177	0.3090	0.5392	0.1640 to 1.7723

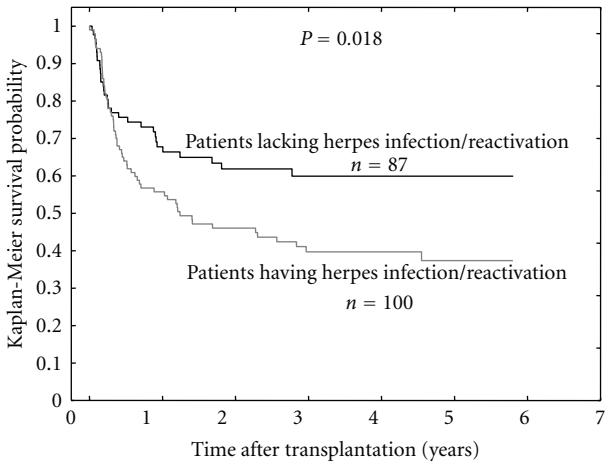


Figure 1: Overall survival in the groups of patients having and lacking *herpes* virus (CMV and/or EBV and/or HHV6) reactivations/infections.

CMV antigen, were incubated with the recipient or donor plasma. Bound IgG or IgM was detected with peroxidase labeled anti-IgG and anti-IgM antibodies by the addition of the color substrate and reading by spectrometry. Results were interpreted as seropositive or seronegative as per the manufacturer's instructions.

DNA was extracted from peripheral blood (QiAmp Blood Kit; Qiagen, Hilden, Germany) according to the manufacturer's instructions. The numbers of CMV, EBV, and HHV6 DNA copies in peripheral blood cells were determined using real-time PCR and Light Cycler II (Roche, Mannheim, Germany). The sequences of the PCR primers and the probe were selected from the *BALF5* region of EBV, the *US17* region of CMV, and the *U67* region of HHV6. PCRs were performed as described by Jaskula et al. [12].

2.1. Statistical Analysis. Statistical analysis was performed using the CSS Statistica for Windows (version 10.0) software (Stat-Soft Inc., Tulsa, OK). Univariate analyses were performed by the Fisher exact test. Logistic regression was used for the multivariate analysis, and a log-rank test to analyze the survival probability. Differences between samples were considered to be significant when $P < 0.05$ and those between 0.05 and 0.1 were indicative of a trend.

3. Results and Discussion

The presence of >100 CMV, EBV, and HHV6 DNA copies per 10^5 blood cells (clinically significant [12, 13]) was detected in 29%, 24%, and 18% of patients, respectively. Sixty out of 100 patients having during the observation period one or more reactivation events of one or more examined herpes viruses died. The mortality rate was lower in the group of patients lacking reactivations/infections (32 out of 87 patients), which resulted in a better five-year survival (59% versus 37%, $P = 0.018$, Figure 1).

Table 4: Multivariate analysis of risk factors for CMV reactivation/infection.

Variable	Coefficient	P value	Odds ratio	95% CI
Recipient CMV IgG seronegativity	−0.0761	0.9224	0.9267	0.2000 to 4.2929
Donor/recipient HLA mismatch	**1.2525**	**0.0155**	**3.4992**	**1.2695 to 9.6446**
R CMV IgG+/D CMV IgG−	**3.1370**	**0.0227**	**23.0340**	**1.5491 to 342.4999**
Unrelated donor	0.0021	0.9965	1.0021	0.3904 to 2.5722
aGvHD > 1	0.5363	0.1755	1.7096	0.7870 to 3.7141
Recipient age > 16 years	**2.2890**	**0.0072**	**9.8650**	**1.8606 to 52.3036**

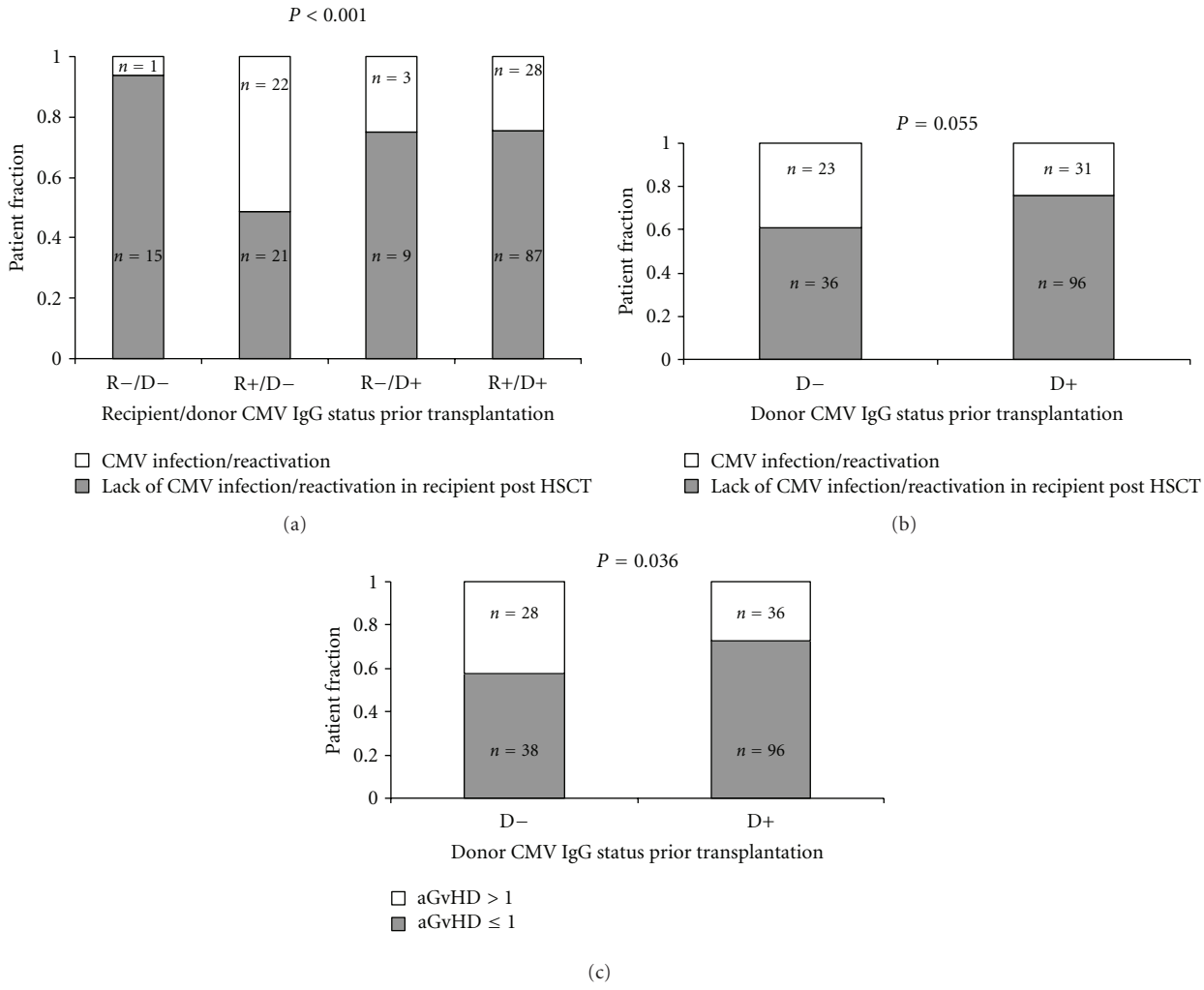


Figure 2: CMV reactivation/infection with respect to donor/recipient CMV serology (a) and donor CMV IgG status independently of the serostatus of recipients (b). Acute GvHD in patients transplanted from CMV IgG negative and CMV IgG positive donors (c) (R: recipient, D: donor, "+": CMV IgG positive, and "−": CMV IgG negative).

Patients receiving transplantation from the CMV IgG seronegative donors tended to suffer more frequently from CMV infection/reactivation after HSCT as compared to those grafted from CMV seropositive donors (23/59 versus 31/127, $P = 0.055$, Figure 2(b)). This association was valid for seropositive and seronegative recipients. However, the highest risk of CMV reactivation was when seropositive recipients were transplanted from the seronegative donors (22/43 versus 32/143, $P < 0.001$ Figure 2(a)). In contrast, CMV negative serostatus of both the donor and the recipient was associated with the lowest rate of the CMV reactivation (1 out of 16 patients) as compared to other recipient (R)/donor (D) CMV IgG serostatus relations, being 22/43 versus 28/115 versus 3/12, ($P < 0.001$) for R+/D−, R+/D+, and R−/D+, resp. (Figure 2(a)). We also found that aGvHD (grade > I) was more frequently seen in patients receiving grafts from IgG negative donors (28/66 versus 36/132, $P = 0.036$, Figure 2(c)). However, donor

Table 5: Univariate analysis of risk factors for CMV reactivation/infection event(s) in group of SIB and MUD HLA match patients and in group of MUD HLA mismatch patients.

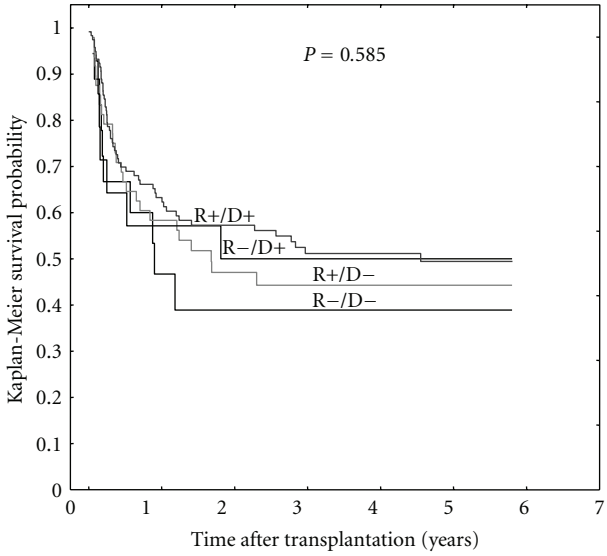
Variable	Optimally matched group (SIB+ 10/10 HLA matched) of patients			MUD HLA mismatched group of patients		
	CMV absence Infection/reactivation until 1 year post HSCT	CMV presence Infection/reactivation until 1 year post HSCT	*P* value	CMV absence Infection/reactivation until 1 year post HSCT	CMV presence Infection/reactivation until 1 year post HSCT	*P* value
Source of HSCT						
PBPC	95	30	1.000	17	18	0.232
BM	18	6		3	0	
Conditioning regimen						
Absence of ATG and Campath	33	5				
ATG	60	22	0.167	19	14	0.170
Campath	20	9		1	4	
RIC	**53**	**23**	**0.087**	7	7	1.000
Myeloablative	60	13		13	11	
Donor CMV IgG						
CMV IgG−	26	12	0.273	10	11	0.532
CMV IgG+	86	24		10	7	
Recipient CMV IgG						
CMV IgG−	18	2	0.160	6	2	0.238
CMV IgG+	95	34		14	16	
Donor-recipient IgG CMV serology						
R−/D−	**10**	**0**		**5**	**1**	
R−/D+	8	2	**0.032**	1	1	**0.18**
R+/D−	**16**	**12**		**5**	**10**	
R+/D+	78	22		9	6	
R−/D−, R−/D+, R+/D+	96	24	**0.015**	15	8	**0.096**
R+/D−	**16**	**12**		**5**	**10**	
Donor/recipient gender						
Male to male, female to female, and male to female	67	23	0.698	13	14	0.485
Female to male	46	13		7	4	
Donor gender						
Male	67	23	0.569	8	9	0.746
Female	46	13		12	9	
Recipient gender						
Male	59	21	0.698	12	6	0.112
Female	54	15		8	12	
Recipient age						
≤16	11	1	0.290	9	1	**0.009**
>16	102	35		**11**	**17**	
aGvHD						
aGvHD ≤ grade I	88	23	0.123	9	7	0.752
aGvHD > grade I	25	13		11	11	

serostatus did not affect the survival of HSCT recipients (Figure 3).

In addition to the factors associated with the serostatus of donors and recipients, a lack of optimal donor/recipient HLA matching was associated with a higher risk of grade > I aGvHD (25/44 versus 40/156, $P < 0.001$) and with a higher rate of CMV reactivation/infection (18/38 versus 36/149, $P = 0.008$). CMV reactivation was also more frequently

Table 6: Multivariate analysis of risk factors for CMV infection/reactivation in group of SIB and MUD HLA match patients and in group of MUD HLA mismatch patients.

Variable	Optimally matched group (SIB+ 10/10 HLA matched) of patients				MUD HLA mismatched group of patients			
	Coefficient	*P* value	Odds ratio	95% CI	Coefficient	*P* value	Odds ratio	95% CI
aGvHD > 1	0.7265	0.1015	2.0679	0.8667 to 4.9336	0.0185	0.9816	1.0187	0.2113 to 4.9106
Recipient CMV IgG seronegativity	0.9058	0.2601	2.474	0.5113 to 11.9711	0.5751	0.6126	1.7773	0.1918 to 16.4703
R CMV IgG+/D CMV IgG−	**0.9819**	**0.0374**	**2.6695**	**1.0587 to 6.7314**	**1.6719**	**0.0776**	**5.3222**	**0.8313 to 34.0733**
RIC conditioning regimen	−0.5655	0.1745	0.5681	0.2511 to 1.2849	0.30101	0.7333	1.3513	0.2391 to 7.6375
Recipient age > 16 years	1.2945	0.2318	3.6492	0.4371 to 30.4624	**3.1026**	**0.0148**	**22.256**	**1.8360 to 269.7835**



R−/D−, $n = 18$
R+/D−, $n = 48$
R−/D+, $n = 118$
R+/D+, $n = 14$

Figure 3: Survival of HSCT patients in the groups stratified according to CMV donor-recipient serostatus constellation (R: recipient, D: donor, "+": CMV IgG positive, and "−": CMV IgG negative).

seen in patients who were over 16 years old at the time of transplantation (52/165 versus 2/22, $P = 0.042$, Table 2) and in those having CMV IgG antibodies before transplantation (50/159 versus 4/28, $P = 0.073$, Table 2).

Multivariate analysis devoted to the evaluation of the risk factors of aGvHD showed that unrelated donor (OR = 2.591, $P = 0.036$) transplantation and HLA mismatch (OR = 2.361, $P = 0.042$) appeared as independent and significant factors associated with aGvHD grade > I (Table 3). In spite of the univariate results multivariate analysis did not confirm the role of CMV reactivation and donor serology as independent factors associated with aGvHD (Table 3).

The next statistical approach was to validate factors associated with CMV reactivation. For that also a multivariate analysis was calculated employing factors as follows: recipient IgG serology, donor-recipient HLA mismatch, transplantation recipient in CMV IgG positive/donor CMV IgG negative serology, type of donors, recipient age, and aGvHD. Among the above factors donor-recipient HLA mismatch (OR = 3.499, $P = 0.016$), recipient CMV IgG positive/donor CMV IgG negative serology status constellation (OR = 23.030, $P = 0.023$), and recipient age over 16 years (OR = 9.865, $P = 0.007$) were found to be significant risk factors of CMV reactivation (Table 4).

To further analyze the significance of CMV serology as a risk factor of CMV reactivation similar to that above, analysis was performed for groups consisting of SIB and MUD 10/10 matched and MUD not optimally matched (Tables 5 and 6). On a group basis independently of the CMV serostatus of donor-recipient pairs, sibling transplantations and those from unrelated donors matched 10/10 at allele level had a similar rate of CMV reactivation (17/78 versus 19/71, P = ns). Notably, the rate of CMV reactivation was higher in patients grafted from donors accepted at the lower level of matching than 10/10 (18/38 versus 36/149, $P = 0.008$). Also when we considered separately the optimal match group (SIB + MUD) the highest risk of CMV reactivation was observed when donors were negative but recipients were positive (12/28 versus 24/120, $P = 0.015$). In MUD HLA mismatched recipients a tendency to the association seropositivity of recipients with concomitant seronegativity of donors with the CMV reactivation/infection was observed (10/15 versus 8/23, $P = 0.096$). Notably in the MUD HLA mismatch group recipient age >16 years was a risk factor for CMV reactivation (17/28 versus 1/10, $P = 0.009$, Table 5). There were no significant associations between aGvHD and variables considered in this paper in the optimally matched group (SIB + 10/10 HLA matched) and in the MUD HLA mismatched group of patients.

Multivariate analysis results of patients optimally matched and separately those not optimally matched were similar and revealed that among factors analyzed for the risk of CMV reactivation seropositivity of recipients with

concomitant seronegativity of donors plays an independent role (OR = 2.670, $P = 0.037$) for optimally matched and as tendency in HLA mismatched patients (OR = 5.322, $P = 0.078$, Table 6).

4. Conclusions

The information provided in the present paper shows that IgG negativity in donors favors the outcome of HSCT only when recipients are also CMV IgG negative. The worst is when donor IgG CMV negativity is confronting IgG CMV positivity in recipients. This confirms the importance of CMV IgG positivity likely associated with the immune competence of donors [3–5], which is of a special value in seropositive patients, very likely having CMV in a latent form [6]. Therefore, when recipients are IgG CMV positive the immune competence of donors is required to reduce the risk of CMV reactivation. This observation can be used as one of the factors that should be considered during donor selections for an optimal post-HSCT outcome.

Acknowledgment

This work was supported by Grant N R13 0082 06 from the Polish Ministry of Science and Higher Education.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 136087, 13 pages
doi:10.1155/2012/136087

Research Article

Human Leukocyte Antigen Profiles of Latin American Populations: Differential Admixture and Its Potential Impact on Hematopoietic Stem Cell Transplantation

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Received 6 August 2012; Accepted 12 October 2012

Academic Editor: Colette Raffoux

The outcome of hematopoietic stem cell transplantation (HSCT) is shaped by both clinical and genetic factors that determine its success. Genetic factors including human leukocyte antigen (HLA) and non-HLA genetic variants are believed to influence the risk of potentially fatal complications after the transplant. Moreover, ethnicity has been proposed as a factor modifying the risk of graft-versus-host disease. The populations of Latin America are a complex array of different admixture processes with varying degrees of ancestral population proportions that came in different migration waves. This complexity makes the study of genetic risks in this region complicated unless the extent of this variation is thoroughly characterized. In this study we compared the HLA-A and HLA-B allele group profiles for 31 Latin American populations and 61 ancestral populations from Iberia, Italy, Sub-Saharan Africa, and America. Results from population genetics comparisons show a wide variation in the HLA profiles from the Latin American populations that correlate with different admixture proportions. Populations in Latin America seem to be organized in at least three groups with (1) strong Amerindian admixture, (2) strong Caucasian component, and (3) a Caucasian-African gradient. These results imply that genetic risk assessment for HSCT in Latin America has to be adapted for different population subgroups rather than as a pan-Hispanic/Latino analysis.

1. Introduction

Hematopoietic stem cell transplantation (HSCT) is a curative therapy used for the treatment of malignant and nonmalignant hematologic diseases, congenital immune deficiencies, solid tumors, and metabolic diseases [1]. Its outcome is shaped not only by clinical factors [2], but also by the genetics of the patient-donor pair [3]. Apart from the normal compatibility defined by the human leukocyte antigen (HLA) system [4, 5], variation in several genetic systems is thought to have an impact on the complications experienced by patients that undergo this procedure [6].

Graft-versus-host disease (GVHD) is a major complication affecting the success of the transplant and the survival of the patients. Despite the fact that most transplants are performed with high levels of compatibility in terms of HLA, a significant proportion of these transplants is affected by GVHD. Apart from clinical factors [7], a genetic component for GVHD other than HLA has been pointed out as responsible for the occurrence of GVHD in 10/10 HLA compatible patient-donor pairs [8, 9]. Moreover, an ethnicity-driven risk of suffering GVHD after HSCT has been identified [10, 11]. However, these studies focused on "island" populations and broader populations with low admixture proportions, and further studies in admixed populations are lacking.

Latin America is a region where the most dramatic human migrations have taken place, from the early north-eastern Asian bands of hunter-gatherers that conquered the

last continent humanity had expanded to [12], through the 16th and 17th centuries European colonization and bringing of sub-Saharan African (SSA) slaves [13], to the latest waves of immigrants from all over the world in the last two centuries [14]. This complex population history makes present Latin American Populations (LAP) possibly the most ethnically diverse on the planet. This genetic diversity is thus likely to impact the effect of genetics on HSCT and hence it is necessary to understand it in order to be able to interpret genetic association studies in this and other medical fields.

In this study, we used population genetics tools to compare the HLA profiles of 31 LAP and 61 ancestral populations in order to characterise their diversity and classify them according to their genetic makeup.

2. Materials and Methods

2.1. Population Samples. A selection of 92 populations from Latin America, Iberia, Italy, and sub-Saharan Africa with available DNA-based typing data for HLA-A and HLA-B allele groups was made and their details are shown in Table 1. Of these, 31 LAP were defined as populations living in this region that were not classed as Amerindian. Population samples from LAP that have emigrated to the USA and Spain were also included in the analyses.

The remaining 61 populations are native population samples from the three ancestral regions that have contributed majorly to the Latin American gene pool: Amerindians (22 populations), Caucasians from Europe (Iberians and Italians, 19 populations), and SSA (20 populations). In the Caucasian population group, a sample of Italians was selected to complement the Iberian populations in view of the important immigration from this country into some LAP. In total, the population array included 384,446 chromosomes. HLA frequency data was extracted from journal articles and/or the Allele Frequencies database website [15]. The approximate geographic location for the LAP is shown in Figure 1.

2.2. Database Construction. A database containing the frequencies for 47 HLA-A and HLA-B allele groups from the 92 populations that were selected was built. When the available data were at high resolution, the data were reduced to two-digit allele groups. The database was constructed on the Multi-Variate Statistical Package (MVSP, Kovach Computing Services, Anglesey, Wales) and was independently checked for accuracy.

2.3. Population Comparisons. The HLA-A and HLA-B profiles of the 92 populations were analysed by clustering analysis and Principal Coordinates Analysis (PCO), both based on Euclidean distances. The clustering analysis was performed dually and dendrograms were generated for both analyses. The clustering method was based on minimum variance of squared Euclidean distances with a randomized input order. The Eigenanalysis for the PCO was performed at an accuracy of 1E-7 and axes were extracted according to Kaiser's rule [66].

Additionally, three ancestry-specific HLA allele groups were compared between population subgroups in order to illustrate the relative contribution of each ancestral population across LAP.

3. Results

3.1. Clustering Analysis. A dendrogram based on squared Euclidean distances was generated by the comparison of 47 HLA-A and HLA-B allele group frequencies present in the 61 ancestral populations and the 31 LAP. The results for this analysis are shown in Figure 2(a). The first split is between the Amerindian cluster and the Caucasians and SSA, which is consistent with higher differentiation of these populations. The next split is between the SSA and the Caucasian and most of the LAP.

A closer look at the clusters shows that there is a correlation between the geographic location of the ancestral populations and the branching within the clusters. Within the Amerindian cluster, 4 groups form a South American lowland group, a South American Andean group, a Central American group, and a more distinct North American-Alaskan group. A similar correlation is seen within the SSA cluster: western Africans split from the southern, eastern and central African populations. Some of the LAP cluster with the Amerindians, such as the Peruvian mestizos from Arequipa, or with the SSA, as the Cuban Mulattos and the Afro-Brazilians from Paraná. However, 90% of the LAP cluster within a distinct group which includes the Iberians and Italians.

The LAP-Iberian+Italian cluster splits further in distinct subgroups. Most of the Spanish populations, and minority populations from Spain, cluster in their own groups. Also, there is a broad group that clusters all of the remaining Brazilian and Cuban populations, and another one that clusters the Portuguese, Italian, and Argentinians from La Plata, the region of Cuyo, and Buenos Aires. Finally, the last cluster includes the admixed populations from Mexico, Colombia, Venezuela, Costa Rica, as well as the South American immigrants to Madrid and the Mexican and pan-Hispanic samples from the United States.

A dual-clustering method was applied to the dataset in order to identify the groups of alleles that are most variable between the populations. The results from this analysis are shown in Figure 2(b). Clusters of signature ancestry markers can be identified, such as frequent Amerindian input allele groups (HLA-A*68, -B*15, -A*31, -B*48, -B*40, and -B*39), frequent Iberian and Italian Caucasian markers (HLA-A*03, -A*29, -B*07, -B*44, -A*01, and-B*51), and frequent alleles that are evidence of SSA genetic input (HLA-A*30, -A*23, -B*53, -B*58, -B*45, and -B*42).

3.2. Principal Coordinates Analysis. The results from the PCO are shown in Figure 3. Firstly, the ancestral populations (Figure 3(a)) show a clear location. The first PC correlates with the Amerindian-non-amerindian split seen in the cluster analysis, whereas the second split (SSA-Caucasians) correlates with the second PC. Amerindian populations show

Table 1: Summary and details of the populations included in the analyses.

Code	Population	Size ($2n$)	Reference
Amerindians			
ArgCh	Argentinian Chiriguano	108	[15]
ArgET	Argentinian Eastern Toba	270	[16, 17]
ArgRT	Argentinian Toba from Rosario	172	[15]
BolA	Bolivian Aymara	204	[18]
BolQ	Bolivian Quechua	160	[19]
BraT	Brazilian Terena	120	[20]
GuaM	Guatemalan Maya	264	[21]
MexChT	Mexican Tarahumara from Chihuahua	88	[22]
MexMT	Mexican Tarasco from Michoacán	260	[23]
MexOMx	Mexican Mixe from Oaxaca	110	[24]
MexOMxt	Mexican Mixtec from Oaxaca	206	[24]
MexOZ	Mexican Zapotec from Oaxaca	180	[24]
MexTH	Mexican Teenek from Huasteca region	110	[25]
ParGua	Paraguayan Guaraní	80	[26]
PerLC	Peruvian Lama	166	[15]
PerTU	Peruvian Uro	210	[27]
VenPMB	Venezuelan Bari	110	[28]
VenSPY	Venezuelan Yucpa	146	[29]
USAYN	Alaska Yupik Natives	504	[30]
USAAI	Arizona Gila River Indian	984	[31]
USAPi	Arizona Pima	200	[28]
USSDS	South Dakota Lakota Sioux	604	[32]
LAP			
ArgBA	Argentinians from Buenos Aires	2,432	[15]
ArgCY	Argentinians from Cuyo Region	840	[15]
ArgLP	Argentinians from La Plata	200	[15]
Bra	Brazilians	216	[28]
BraBH	Brazilians from Belo Horizonte	190	[33, 34]
BraMG	Brazilians from Minas Gerais	2,000	[15]
BraPAB	Afro-Brazilians from Paraná	154	[35]
BraPCaf	Brazilian Cafuzo from Paraná	638	[35]
BraPCau	Brazilian Caucasian from Paraná	5,550	[35]
BraPMul	Brazilian Mulatto from Paraná	372	[35]
BraPS	Brazilians from Pernambuco State	202	[36]
BraSP	Brazilians from Sao Paulo	478	[15]
CCVP	Costa Ricans from the Central Valley	364	[37]
ChilS	Chileans from Santiago	140	[15]
Col	Colombians	1,122	[38]
ColMed	Colombians from Medellin	1,852	[39]
CubMx	Cubans (mixed)	378	[40]
CubMu	Cuban Mulattos	84	[33, 34]
CubWh	Cuban Whites	140	[33, 34]
MadAm	Latin American immigrants in Madrid	346	[41]
MexGM	Mexicans from Guadalajara	206	[42]
MexCM	Mexicans from Mexico City	242	[43]
MexSM	Mexicans from Sinaloa	112	[43]
MexPM	Mexicans from Puebla	198	[43]
ParM	Paraguayans	100	[44]

Table 1: Continued.

Code	Population	Size ($2n$)	Reference
PerA	Peruvians from Arequipa	336	[45]
USHis	US Hispanics	468	[15]
USHis2	US Hispanics	3,998	[46]
USHisO	US Hispanics	3,160	[47]
USMex	US Mexicans	1,106	[48]
VenCVM	Venezuelans from Caracas, Valencia, and Maracaibo	192	[15]
Iberians and Italians			
BasA	Basques from Arratia Valley	166	[49]
BasG	Basques from Guipuskoa	200	[50]
C&L	Castilians	3,880	[51]
CatG	Catalonians from Girona	176	[50]
And	Spanish from Andalucía	198	[15]
AndG	Spanish Gypsy from Andalucía	198	[15]
Ibi	Spanish from Ibiza	176	[52]
Maj	Majorcans	814	[52]
MajJD	Majorcans of Jewish descent	206	[52]
Min	Minorcans	188	[52]
Mur	Murcians	346	[53]
NCab	North Cabuernigo	190	[49]
NCant	North Cantabrians	166	[49]
PasV	Spanish from Pas Valley	176	[49]
AzoTI	Azoreans from Terceira Island	260	[15]
Ita	Italians	318,622	[54]
PorC	Portuguese from central Portugal	1,124	[15]
PorP	Portuguese from Porto	15,874	[15]
PorF	Portuguese from Faro	2,484	[15]
SSA			
CamBa	Cameroon Bamileke	154	[55]
CamBe	Cameroon Beti	348	[55]
CamYa	Cameroon Yaounde	184	[56]
CapVNW	Cape Verdeans from NW island	124	[57]
CapVSE	Cape Verdeans from SE island	124	[57]
CAFMP	Pygmy from the Central African Republic	72	[58]
GuiB	Guineans	130	[57]
Ken	Kenyans	288	[28]
KenL	Kenyans-Luo	530	[59]
KenN	Kenyans-Nandi	480	[59]
MalB	Mali Bandiagara	276	[59]
Moz	Mozambicans	500	[60]
Rwa	Rwandans	560	[61]
STIF	Sao Tome Islanders (Forro)	132	[62]
SenNM	Senegalese (Madenka)	330	[63]
SAB	Black South Africans	400	[64]
Sud	Sudanese	400	[15]
UgaK	Ugandan from Kampala	350	[65]
ZamL	Zambians from Lusaka	88	[59]
ZimHS	Zimbabwe Harare Shona	460	[28]
Total	92	384,446	

Figure 1: Map showing the approximate location of the LAP included in the study.

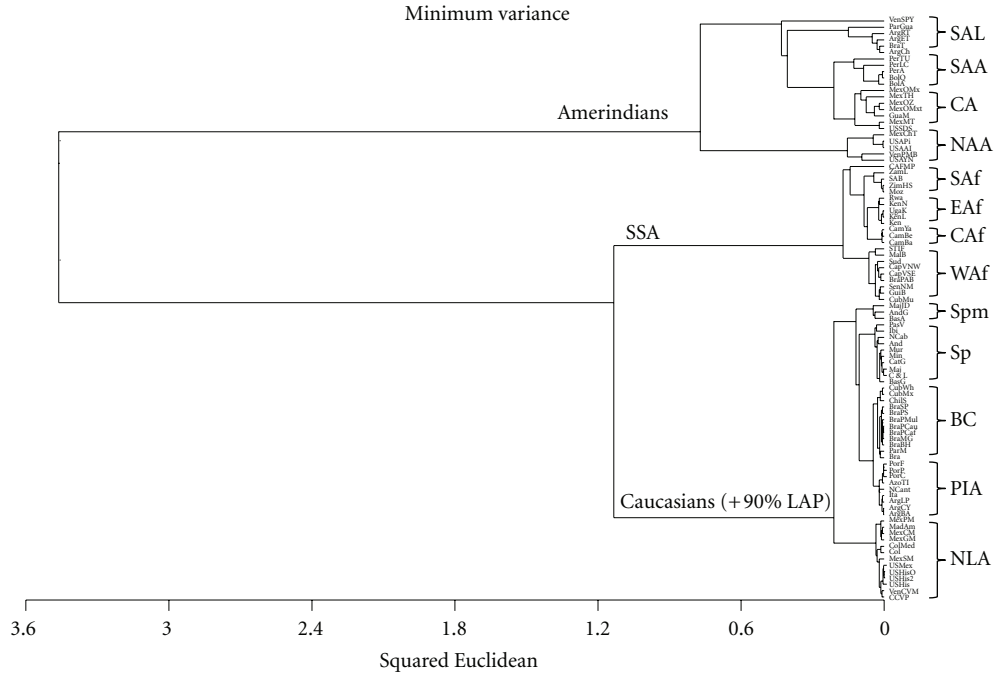
a much more dispersed array and higher distances from the Caucasians and the SSA, which is in accordance with their genetic history being shaped by the colonization of the last continent after the out-of-Africa migrations and successive bottlenecks in this process [67]. Interestingly, SSA that have been in closer genetic and geographic contact with the Caucasians, such as the Sudanese and the Cape Verdeans, are closer to these populations, whereas the southernmost Africans lie on the upper left extreme of the PCO map. Likewise, the North American Amerindians tend to be closer to the Alaskan Natives, who have been shown to be genetically different from the Amerindians of more southern regions [30].

When the LAP are included in the analysis (Figure 3(b)), the results show that LAP are located on a wide arch that connects the three ancestral populations. This arch stretches from the Peruvian mestizos from Arequipa, which appear deep into the Amerindian region, to the Afro-Brazilians from Paraná, which lie on the periphery of the SSA cluster. In between these populations there is a spectrum of locations for the remaining LAP. It is clear that there are two major regions: one that includes the LAP that lie between the Iberian and Italian populations and the Amerindian region and the others, which lie between the Caucasians and the SSA populations. Moreover, the first group seems to be divided in two subregions: one that clusters populations that lie closely to the Caucasian group (from the Argentinians from Buenos Aires to the Hispanic samples from the US), and the other (from the Mexican population from the US up to the Peruvians from Arequipa) which is dragged more intensely towards the Amerindians. The SSA component in these populations seems to be reduced, although not absent (see below). On the other hand, the populations that lie between the Caucasians and the SSA samples also cluster closely to the Iberians and Italians, but show a gradient towards the SSA cluster. This group is composed mainly by Brazilian and Cuban populations.

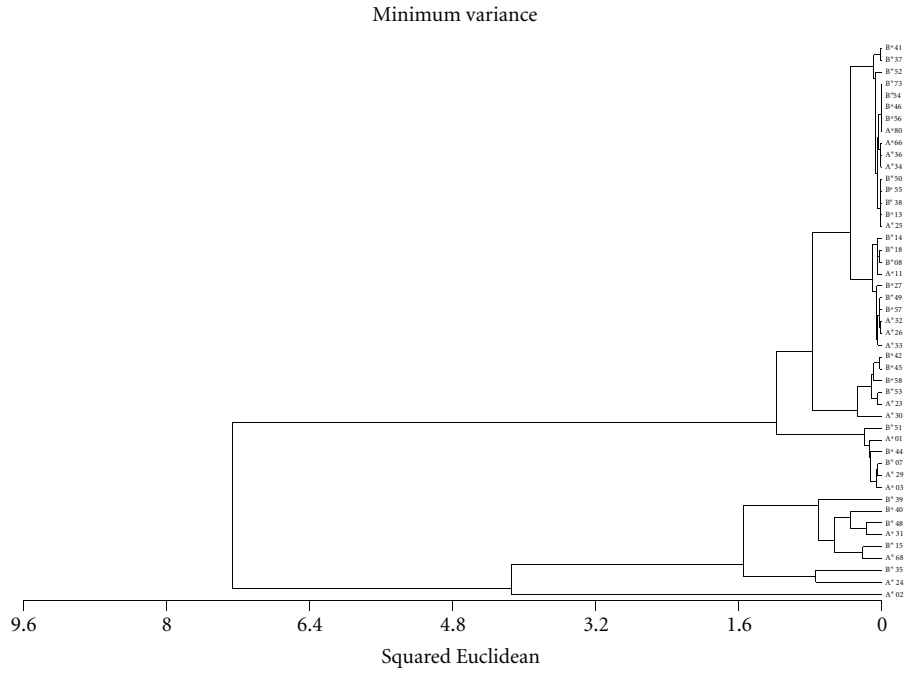
A few populations seem to cluster closely enough to the ancestral populations to be considered part of those clusters. This is the case of the Cuban Mulattos and the Afro-Brazilians from Paraná, the Cuban Whites, the Brazilian Caucasians from Paraná, and the Peruvians of Arequipa. In turn, it must be noted that a population classified as Amerindian, the Argentinian Toba from the city of Rosario, show significant Caucasian admixture and, consequently, lie closer to the admixed Mexicans than the Peruvians do.

3.3. Specific Ancestry Markers. To further illustrate the differential admixture patters present in LAP based on their HLA profile, 3 allele groups which are present in one of the ancestral populations and absent or nearly absent in the other two (HLA-A*25, -B*42, and -B*48) were selected in order to evaluate their frequency among the LAP groups (Figure 4). As seen in Figure 4(a), HLA-A*25, a common allele group in western Europe and virtually absent in Amerindian and SSA populations, is present more frequently in the admixed populations with strong Caucasian component (i.e., those that lie closest to the Caucasians on the Caucasian-Amerindian axis of the PCO). Interestingly, some of the ancestral populations classified as Amerindian show evidence of Caucasian admixture as demonstrated by the presence of HLA-A*25 alleles in their gene pool.

Figure 4(b) shows the frequency of the SSA allele group HLA-B*42 in the 3 LAP subgroups. It is evident that these

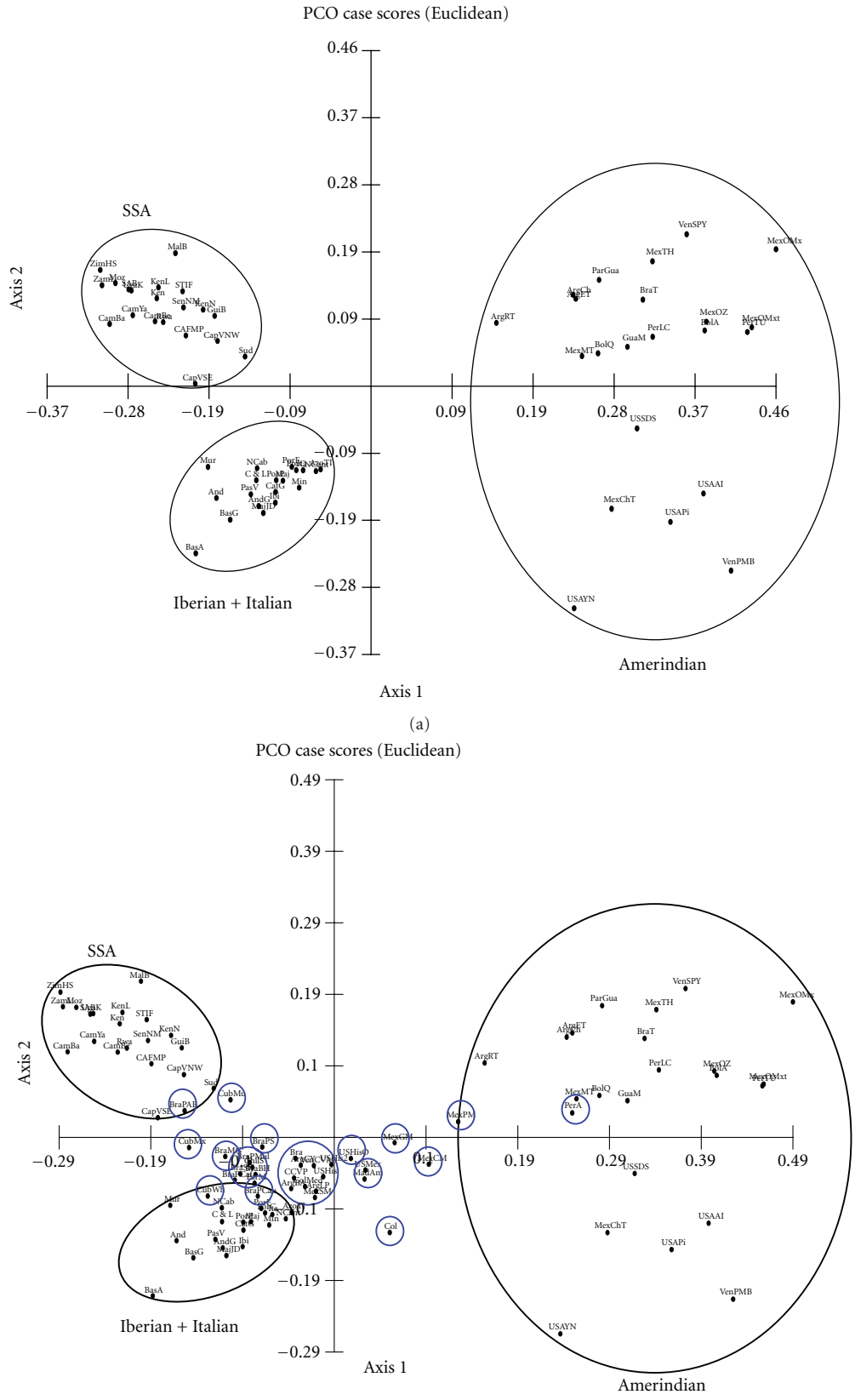


(a)



(b)

Figure 2: Cluster analysis based on 47 HLA-A and HLA-B allele group frequencies among 31 LAP and 61 ancestral populations. (a) Dendrogram showing the clustering of the 92 populations. (b) Dendrogram showing the dual-clustering of HLA allele groups in the dataset. SSA: Sub-Saharan Africans; SAL: South American Lowlanders; SAA: South American Andeans; CA: Central Americans; NAA: North Americans and Alaskans; SAf: Southern Africans; EAf, Eastern Africans; CAf: Central Africans; WAf: Western Africans; Spm: Spanish minorities; Sp: Spanish; BC: Brazilians and Cubans; PIA: Portuguese, Italians, and Argentinians; NLA: Northern Latin Americans.



(a)

(b)

Figure 3: Principal coordinates analysis (PCO) based on the frequencies of 47 HLA-A and HLA-B allele groups in 31 LAP and 61 ancestral populations. (a) PCO map of the first 2 principal components (57.7% cumulative variance) for 61 ancestral populations from sub-Saharan Africa (SSA), America, and Europe. (b) PCO map showing the first 2 principal components (56.7% cumulative variance) for 31 LAP (blue) and 61 ancestral populations.

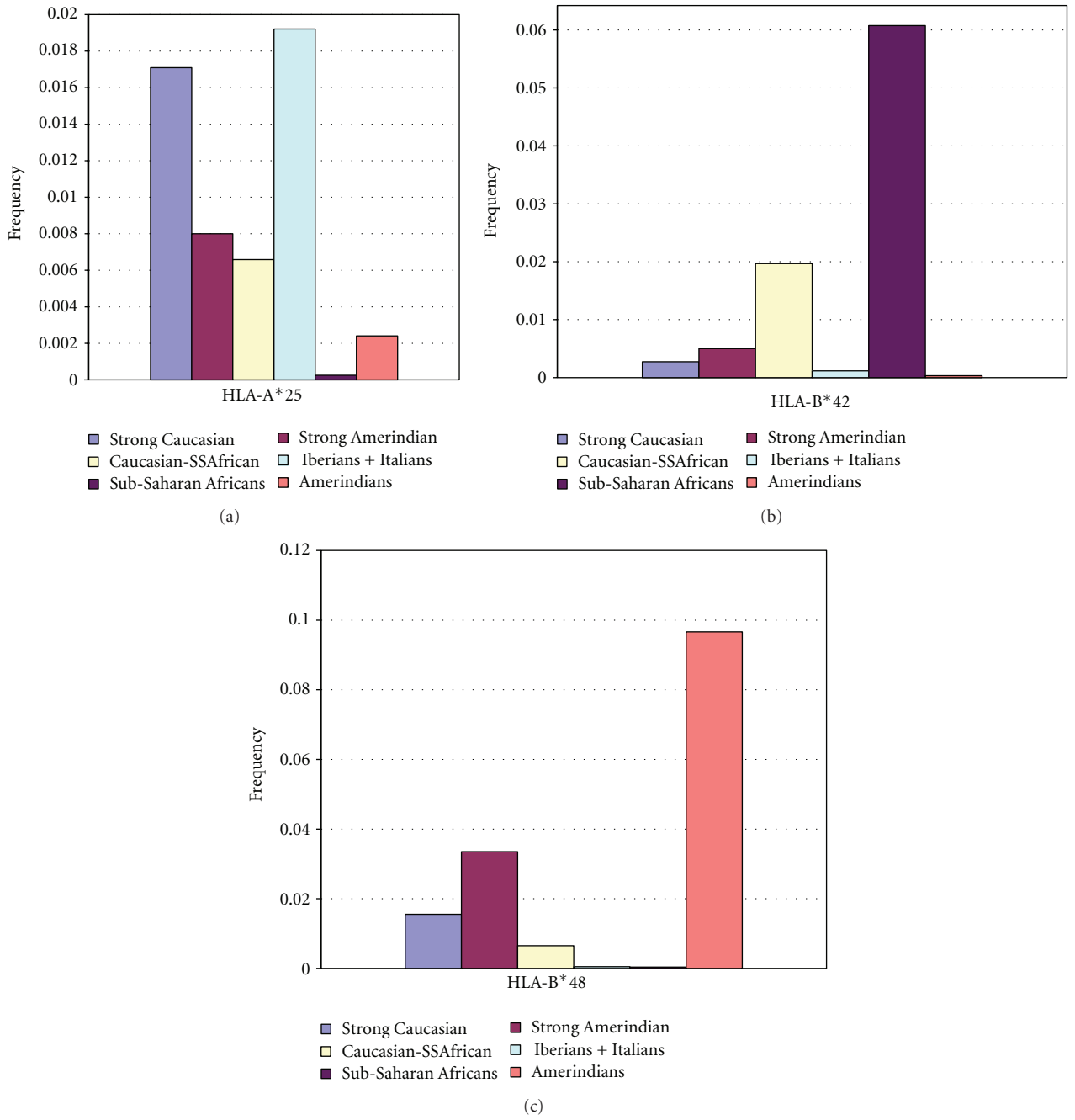


Figure 4: Frequency of ethnic-specific HLA allele groups among three subgroups of LAP and the ancestral populations. (a) Frequency of HLA-A*25 allele group as a Caucasian marker. (b) Frequency of HLA-B*42 allele group as a Sub-Saharan African (SSA) marker. (c) Frequency of HLA-B*48 as an Amerindian marker.

alleles are much more frequent in these populations (mainly, Brazilian and Cuban) than in the rest. However, HLA-B*42 alleles are not absent from other LAP, which may have lower levels of SSA admixture.

Finally, Figure 4(c) shows the average frequency of HLA-B*48 alleles. This group, common in Amerindians and nearly absent from SSA and Iberian and Italian populations, is more strongly represented in the populations that form the bridge between the Amerindian and Caucasian regions in the PCO.

4. Discussion

The results obtained after comparing 47 HLA-A and HLA-B allele groups among the LAP and their ancestral populations show that there is widespread variation between the genetic profiles of these admixed or exported populations. In the cluster analysis it is clear that most LAP have substantial Caucasian components, with the exception of some populations such as the Peruvians from Arequipa or the Afro-Brazilians from Paraná. This is in agreement with the uneven process

of population replacement and the collapse of many Native American groups that took place throughout the continent.

However, PCO analysis showed that most LAP sit on a wide admixture arch that approaches the ancestral clusters. A few populations fall very close to or in the ancestral clusters, but most are scattered in intermediate regions. Interestingly, population samples that are likely to be a mixture of several LAP, such as those of the USA Hispanic immigrants and the Ibero-American expatriates in Madrid, sit in the center of the distribution. In fact, the heterogeneity of the Hispanic population in the US has been described using other markers [68, 69], showing differential admixture patterns between areas that have received mostly Mexican immigration and those that are predominantly colonized by Caribbean islanders from Cuba and Puerto Rico. In agreement with this, US Mexicans lie slightly closer to the Amerindian side on the PCO and locate between the Mexican populations from the center and the sample from the northern state of Sinaloa. This further illustrates the heterogeneity of the Mexican populations, where a stronger Caucasian component is preserved in the north of the country [43], while the US Mexicans are likely to be a combination of northern and southern Mexican populations.

The stronger Caucasian component in some LAP can be attributed to recent European migration [14], such as that of urban populations from Argentina and some Brazilian populations, or to relatively stronger Caucasian proportions generated at colonial times in areas where Amerindian populations were low at the time of the arrival of Europeans, which is thought to be the case of the Costa Rican Central Valley and the Colombians from Medellin [70, 71].

On the Caucasian-SSA axis of admixture, several Brazilian and Cuban populations can be found. It seems that for these populations, Amerindian admixture is very low or absent. This has been noted by others [72] and it is argued that a dual admixture model is more likely to describe the patterns seen in these populations as opposed to a triple admixture model identified for other LAP. Although not included in our analysis because of the lack of molecular HLA data, serological HLA data from Panama [73] and Puerto Rico [74] suggest that these populations are likely to join this group, whereas the data from Uruguay suggest that its major population would cluster with the strong Caucasian component group [75].

Our study is limited by both the availability of population data and the need to use HLA allele group data for comparison as opposed to high-resolution allele frequencies or haplotype frequencies. It is likely that an analysis of high-resolution frequencies would give finer results, but it would seriously diminish the number of populations that can be included in the analysis. However, the use of 47 allele groups from the most polymorphic genes in the human genome gives robustness to the analysis.

The effect of ethnicity on complications after HSCT has been suspected for many years [76, 77] but some studies have not shown such association [78]. Hence, there is growing interest in unraveling the genetic-ethnic component of GVHD in HLA-compatible HSCT. Currently, there is a project within the International Histocompatibility Working Group that aims at analyzing the risk of GVHD after HSCT in unrelated donor pairs according to the ethnic origin of both patients and donors, based on previous findings in sibling transplantations in isolated and general populations of certain countries [10]. Preliminary results in a cohort of unrelated transplants showed that Hispanic pairs have high risks of mortality and acute GVHD (grades III-IV) only second to African American pairs. Moreover, Hispanic-Hispanic pairs had the highest risk of relapse [79]. Both analyses were carried out having Asian/Pacific (mostly Japanese) ethnically matched pairs as the reference group. These findings suggest that ethnic heterogeneity in the Hispanic population may be playing a role on the risk of complications after HSCT, and the complexity of the admixture patterns illustrated in this study and others is likely to account for much of this variation. Also, ethnicity has been associated with other complications after HSCT such as chronic GVHD [80]. Moreover, an increased risk of complications has been reported specifically for Hispanic groups in North America when compared to other ethnicities in terms of survival [81] and treatment failure [82].

It is likely that the evidence for differential outcome in different ethnic groups could be explained, at least in part, by differences in allele frequencies in genes that are relevant to the immune response and that show variable interethnic polymorphism, such as the cytokine genes [83]. Moreover, polymorphisms in other genes such as those that intervene in drug metabolism or drug targets may play a role in the way patients from different ethnicities respond to treatment in HSCT, especially in admixed populations [84, 85].

LAP show widespread variation in their genetic profiles, and this complicates genetic association studies made in these populations. There is noticeable variation not only between regions and countries, but also between areas of the same country [43, 86]. Furthermore, the presence of minority populations of different ethnic composition adds to the complexity of population stratification in Latin America. Additionally, many populations remain to be studied. If an ethnic component is to be used as one prognostic factor affecting the risk of complications after HSCT, the application of this concept in Latin American populations will have to take into account the great diversity found among the different populations derived from this region and the different population subgroups generated by different admixture histories. Consequently, there is need of a more detailed understanding of the genetic profiles of the LAP, in order to be able to accurately stratify genetic risk in HSCT.

It is also important that a better definition of individual ancestry in LAP is reached in view of the evident limitations of both self-reported [87] and researcher-assigned ethnicities [41, 88]. To this purpose, the use of a more objective assignment based on ancestry markers [69] is likely to increase the accuracy of the information derived from these studies. Hopefully, a finer characterization of the risk of complications after HSCT in LAP will help foresee these complications and increase the access and success of transplantation in these populations.

Acknowledgments

This work was supported by grants from the University of Costa Rica and the Costa Rican National Council for Scientific and Technologic Research (CONICIT).

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 695018, 8 pages
doi:10.1155/2012/695018

Review Article

Unrelated Hematopoietic Stem Cell Donor Matching Probability and Search Algorithm

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Received 9 August 2012; Revised 4 October 2012; Accepted 8 October 2012

Academic Editor: Andrzej Lange

In transplantation of hematopoietic stem cells (HSCs) from unrelated donors a high HLA compatibility level decreases the risk of acute graft-versus-host disease and mortality. The diversity of the HLA system at the allelic and haplotypic level and the heterogeneity of HLA typing data of the registered donors render the search process a complex task. This paper summarizes our experience with a search algorithm that includes at the start of the search a probability estimate (high/intermediate/low) to identify a HLA-A, B, C, DRB1, DQB1-compatible donor (a 10/10 match). Based on 2002–2011 searches about 30% of patients have a high, 30% an intermediate, and 40% a low probability search. Search success rate and duration are presented and discussed in light of the experience of other centers. Overall a 9-10/10 matched HSC donor can now be identified for 60–80% of patients of European descent. For high probability searches donors can be selected on the basis of DPB1-matching with an estimated success rate of >40%. For low probability searches there is no consensus on which HLA incompatibilities are more permissive, although HLA-DQB1 mismatches are generally considered as acceptable. Models for the discrimination of more detrimental mismatches based on specific amino acid residues rather than specific HLA alleles are presented.

1. Introduction

An increasing number of transplantations are now performed with hematopoietic stem cells (HSC) from unrelated volunteer donors. This trend has been largely facilitated by the impressive growth of volunteer donor registries in the last decade: 8 million donors in 2002 and more than 20 million in 2012. The implementation of recipient and donor HLA high resolution genotyping in the clinical practice has clearly contributed to improve the success of transplantation through a better matching [1, 2]. On the other hand the polymorphism of HLA genes turns out to be much higher than anticipated, resulting in larger difficulties in identifying a perfectly matched donor. Because most donors in the Bone Marrow Donor Worldwide (BMDW) registry are of European descent, searches for patients of other ethnic backgrounds have a lower success rate, particularly for those patients with a mixed origin.

HLA matching is commonly based on exons 2 and 3 polymorphism for class I loci and on exon 2 polymorphism for class II loci. The nature of HLA polymorphism with reshuffling of gene segments coding for just a few amino acids has rendered HLA typing a challenging task. The HLA typing techniques currently used in the clinical laboratories often lead to ambiguities because alleles share sequence motifs and because a number of alleles are not resolved by the methods in use. Most typing techniques rely on a locus-specific generic amplification (of one or several exons) which makes it sometimes difficult ot detect whether two polymorphic segments are in *cis* or in *trans* in heterozygous individuals. Furthermore the extension of sequencing techniques to additional exons has disclosed many new alleles, thereby contributing to increase the difficulty of HLA matching. The deleterious impact of single HLA disparities between patient and donor has been largely documented [1–3]. Matching for HLA-A, B, C, DRB1, and DQB1 alleles, a so-called 10/10 match [1–3], and more recently for HLA-DPB1 [2, 4, 5], has been shown to decrease the risk of acute graft-versus-host disease (aGVHD) and mortality after HSCT.

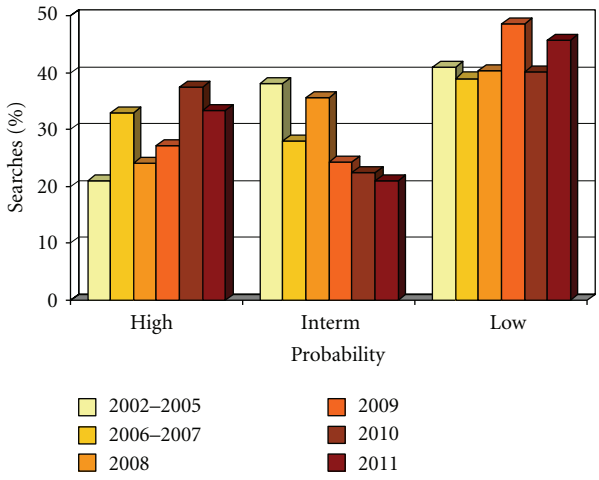

Figure 1: Relative distribution of 1244 high, intermediate, and low probability searches run from 2002 to 2011. The 2002–2005 probability estimates have been reported previously [6].

In 2002 we have introduced at the very start of the search an estimation of the probability to identify a perfectly matched donor, that is, compatible for the HLA-A, B, C, DRB1/B3/B5, and DQB1 loci. The probabilities were classified in 3 categories: high (>95% chance), intermediate (about 50%), and low (<5%). As computed from 350 searches (2002–2005) the positive and negative predictive values were 96% and 88%, respectively [6]. This paper reviews our experience in unrelated HSC donor searches as a follow-up of the search algorithm applied in our laboratory since 2002 [6]. A recent evaluation of the success rate and of the time frame for the identification of a suitable donor as well as the impact of the inclusion of DPB1 matching in the algorithm are presented and compared to those reported by other centers. Criteria that negatively impact the matching probability rate, and HLA-linked parameters that could be taken into account for selecting a mismatched donor, are reviewed. Clinical and functional relevance of HLA disparities is reviewed and possible models for the identification of more detrimental mismatches based on specific amino acid positions are discussed.

2. Search Probabilities

According to the search algorithm initiated in 2002 on a national basis, search probabilities are assigned as high, intermediate, or low based on patients HLA-A, B, C, DRB1/B3/B5,DQB1 haplotypes and on interrogation of the BMDW database [6]. Parameters that are taken into account for the probability assignment are presented in the next section. For each consecutive year the relative ratios of high/intermediate/low probabilities have been computed. All donors were requested by the national registry Swiss Blood Stem Cells (SBSC) and tested by the national reference laboratory for histocompatibility (LNRH). Usually 4–6 donors were requested, taking into account a >20% donor unavailability rate.

As compared to the initial observations of 2002–2005, the ratio of high probability searches has increased from 21% to 33–37% in the last 2 years (Figure 1). However the ratio of low probability searches remained stable around 40%. The absolute increase of the registered donors in BMDW, the implementation of HLA typing data (higher resolution level and additional loci tested) of the newly registered donors, and our increased knowledge on HLA haplotypic frequencies [7–12] have also allowed more precise probability estimates. Indeed the ratio of searches qualified as intermediate probability searches (i.e., the most difficult to assign) has decreased from 38% to 21-22% in the last 2 years (Figure 1). Predictive algorithms based on population HLA allele and haplotype frequencies are used by other centers: Haplogic by the National Marrow Donor Program (NMDP), Optimatch by the German Registry or EasyMatch by the French Registry.

3. Impact of Rare Alleles and Haplotypes on the Search

Based on our experience of the last 10 years, still 2–5% of the patients do have a unique phenotype (not necessarily including a rare HLA variant) that is not represented in the 20 million donors-BMDW registry. A German study based on 2008-2009 searches reported a 3.3% rate [13]. The ratio is expected to be higher for patients of non-European ancestry. In our experience, among 55 patients with 0 donor in BMDW some serotypes occurred more frequently, such as A25, A33, A68, B18, B53, B58, or B72. HLA-DRB1*09:01-, *10:01-, *14:01, *15:02- and *04:02/03/05/06/07/08-positive haplotypes also occurred more frequenly (data not shown).

Criteria that negatively impact the probability to identify a 10/10 compatible donor are summarized in Table 1 and are obviously linked to patients allele/haplotype frequencies [6, 10, 11, 13]. Searches for patients with a rare allele (e.g., B*07:04 or DRB1*11:58 as encountered in patients analysed in our laboratory) have a low probability of success. Even searches for patients with alleles that represent 5–10% of all alleles within a serotype such as B*35:02 or DRB1*13:03, may have a low probability estimate depending on the extended HLA-A, B, DRB1 haplotype. For example matching for A*02:05 will be much easier if the patient has the A2-B50-DR7 haplotype [11] when compared to the A2-B50-DR3 haplotype. Rare alleles are often associated with a well-defined HLA-A-B-DRB1 haplotype, presumably because of a more recent origin of the allele. A few examples are illustrated in Table 2. A most representative case is the A*02:151 allele, initially described as A*9251 [14], that was subsequently confirmed in 17 individuals (http://www.ebi.ac.uk/imgt/hla/): in 13/17 confirmations this allele was identified on the haplotype A*02:151-B*07:02-C*07:02-DRB1*15:01. Consequently, the presence of a rare allele on a given haplotype might not necessarily mean that search will not be successful. Recently sequenced new alleles that differ outside exons 2 and 3 (for class I) and exon 2 (for class II) may also impact on matching probability. A classic example is the DRB1*14:01 versus *14:54 incompatibility. However the clinical relevance of such

Table 1: Parameters that contribute to define a low probability estimate.

HLA, ethnicity, nb donors	Examples and comments
≤3 donors in BMDW	
Non-European ancestry	
Rare[(1)] allele at any locus	A*02:17, B*44:05, DRB1*11:03
Rare B-C association	B*18:01-C*02:02, B*51:01-C*16:02
Rare DRB1-DQB1 association	DRB1*15:01-DQB1*06:03, DRB1*0701-DQB1*03:02
B*15:01, B*18:01, B*27:05, B*51:01-positive haplotypes	Higher risk of C MM
B*35:02/35:03/35:08-positive haplotypes	Higher risk of B*35 allele MM

[(1)] <5% of the alleles included a given serotype.

Table 2: Examples of conserved haplotypes with rare HLA class I alleles.

Rare allele	First assigned	Extended haplotype
A*02:151	2008	A*02:151-B*07:02-C*07:02-DRB1*15:01
A*03:20	2005	A*03:20-B*51:08-C*16:02-DRB1*11:04
A*03:50	2009	A*03:50-B*35:01-C*04:01-DRB1*01:01
A*03:96	2010	A*03:96-B*07:02-C*07:02-DRB1*15:01
A*03:102	2010	A*03:102-B*18:01-C*02:02-DRB1*13:01
B*07:20	1999	A*24:02-B*07:20-C*07:02-DRB1*16:01
B*27:70	2010	A*02:01-B*27:70-C*02:02-DRB1*04:01/04:04
B*51:43	2006	A*02:01-B*51:43-C*14:02-DRB1*04:01
C*05:14	2006	A*02:01-B*51:01-C*05:14-DRB1*04:04
C*15:13	2004	A*02:01-B*51:01-C*15:13-DRB1*04:02

disparities is unknown. Unusual B-C and DRB1-DQB1 [6, 11, 13, 15] associations involving common alleles also lead to low probability searches. In such cases the transplant physician should rapidly consider a 9/10 matched donor with a C or DQB1 mismatch, respectively.

4. Search Algorithm and DPB1 Matching

An outline of the search algorithm as a function of the probability estimate is represented in Table 3. Requesting >2 donors for the high probability searches has also proven to be useful for the rapid identification of a "back-up donor" since the availability rate of selected donors has slightly decreased in the past years. As a major implementation of our initial algorithm [6], we have recently included HLA-DPB1 typing in the algorithm for a fraction of the high probability searches. Selection according to HLA-DPB1 matching was evaluated on 33 patients for whom >1 potential 10/10 matched donor could be identified (January–July 2012). Based on 33 searches we could identify a DPB1 matched donor for 42.4% of the patients (including one DPB1 mismatched pair in rejection direction only), with an average of 2.7 donors tested/patient (range 1–5, 90 donors tested). Although calculated on a limited number of searches that include essentially patients of European ancestry, this is the first evaluation of the success rate of prospective DPB1 typing aiming at the identification of a 12/12 matched donor. If no DPB1-matched donor can be identified, donors can be selected according the T-cell epitope (TCE)3 matching algorithm [4].

5. Efficiency of the Searches

Efficiency of the search is detemined by the likelihood to identify a "matched" donor by testing a "reasonable" number of donors (i.e., in a cost-efficient manner) and by the the time required for the process. Data in the literature on "successful" searches and on search duration are scarce and are difficult to compare mainly because HLA matching criteria vary between the centers. Depending on risk factors such as patient's age, disease stage, or urgency of transplant, a 9/10 matched donor would be considered a suitable donor in center A, but not in center B.

A detailed Dutch study of 212 searches run in 1996–2000 showed that a suitable donor (9-10/10, or <9/10 in 13% cases) could be identified for 69% of the patients with a median search time of 2.5 months [16]. A study from the UK based on 60 unrelated donor searches run in 2005 reported that a 9-10/10 donor could be identified for 72% of the patients with a median time to donor availability of 11 weeks if donor was registered in the UK and of 14 weeks if the donor had to be searched in the international registry [17]. A retrospective evaluation of 549 searches run in 2005 for 23 German transplant centers reported the identification of a 10/10 matched donor for 61.6% of the patients [13]. Overall median search duration was 20 days (7–330), 45 days

Table 3: Unrelated donor search algorithm for high, intermediate, and low probability categories aiming at the identification of 12/12, 10/10, or 9/10 matched donors. This algorithm is based on requesting blood sample from BMDW registries, and histocompatibility testing in the laboratory serving the transplant center(s). Alternatively HLA typing can be performed by the laboratory linked to each registry at the request of the transplant center. Intermediate resolution typing must resolve the main allele groups, for example, B*44:02 versus B*44:03 groups or C*07:01 versus 07:02 groups.

Probability	Steps	Procedure
High	1	Urgent transplant:
		(i) select 2–4 donors (incl. "back-up donor") according to age, sex, CMV status, blood group
		(ii) type for HLA-A, B, C, DRB1/B3/B5, DQB1 at a high resolution level[(1)]
		Nonurgent transplant: consider DPB1 matching (a 12/12 match is possible for >1/3 patients)
	2	(i) type for HLA-A, B, C, and DRB1, DQB1 at an intermediate resolution level
		(ii) type for DRB3 if DRB3 MM risk (i.e. DRB1*13:01 haplotypes)[(1)]
		(iii) if DPB1 matched donor found: complete high resolution typing for all HLA loci
Interm	1	Select 4–6 potential donors and type for HLA-A, B, C, DRB1, and DQB1 at an intermediate level
		>1 potentially matched donor identified: select according non HLA criteria and complete high resolution typing
	2	no matched donor identified and urgent transplant: select according non HLA criteria and complete high resolution typing
	3	no matched donor identified and non-urgent transplant: request another set of 4–6 donors
Low	1	Consider a mismatch early in the search and request 4–6 donors:
		(i) type for HLA-A, B, C, DRB1/B3/B5, DQB1 at an intermediate resolution level
	2	No matched donor identified and urgent transplant:
		(i) select a donor among potential donors with single MM and complete high resolution typing
	3	No matched donor identified and nonurgent transplant:
		(i) request another 4–6 donors and type for HLA-A, B, C, DRB1/B3/B5, and DQB1 at an intermediate resolution level
	4	If no potential donors available in BMDW:
		(i) select donor(s) with a mismatch located at the locus where the patient's rare allele is found
		(ii) if B MM: select donors with B MM associated with same HLA-C (e.g., B35:08 versus B*53:01 or B*13:01 versus B*57:01)
		(iii) if DRB1 MM : select donors with DRB1 mismatches associated with same DQB1 allele (e.g., DRB1*11:03 versus DRB1*12:01)
	5	If no mismatch accepted consider another HSC source (cord blood, haplo-identical donor) or a nontransplant protocol

[(1)] HLA-A, B, C, DRB1, and DQB1 testing is performed by PCR-SSO on microbeads arrays (luminex technology, OneLambda HD reagents) by PCR-SSP (Genovision), and by mono-allelic PCR-SBT (Protrans). HLA-DRB3, DRB5, and DPB1 typing is performed by PCR-SSP.
MM : mismatches.

Table 4: Donor matching grade for 274 consecutive searches run from 1.1.2010 to 31.8.2012.

Category[1]	Nb patients	Nb donors tested	Mean nb don/patient	10/10	9/10[2]	≤8/10 or non evaluable
High	103	331	3.2	102 (99%)	1 (1%)	0
Interm	61	333	5.45	38 (62.2%)	20 (32.8%)	3 (5%)
Low	110	744	6.76	19 (17.3%)	44 (40%)	47 (42.7%)
Total	274	1408	5.14	159 (58%)	65 (23.7%)	50 (18.3%)

[1] For 26 patients classified with a high ($n = 7$), intermediate ($n = 5$), and low ($n = 14$) probability a formal search was not initiated or no donor could be requested or analysed during the same time frame.
[2] DRB3 disparities were counted as a mismatch.

Table 5: Time frame of donor searches run from 1.1.2010 to 31.8.2012 for transplanted patients with different search probability estimates.

Category	Nb patients	Time for donor identification (days)	Time to HSCT (days)	Mean nb donor tested/patient
High[1]	66	54 (20–208)	101 (24–428)	4.92
Interm	30	73 (34–217)	76 (11–170)	5.13
Low	36	83 (33–308)	94 (12–298)	5.05

[1] For 98/99 high probability searches a 10/10 matched donor could be identified with a mean duration of 56 days (20–208), a transplant date was not (yet) available for 18 patients, 5 patients declined transplantation, 6 patients died, 1 relapsed, 1 was transplanted abroad, 1 was transplanted with a haplo-identical donor.

(7–1225), and 477 (2–2870) days in patients groups with high, low and very low search success probabilities, respectively [13]. A recent Austrian study reported that a 9-10/10 (exceptionally a 8/10) matched donor could be identified for 78.3% of the patients (87.7% of European origin) in 2008–2010 searches, with a mean search time of 1.84 months in 2010 [18].

Not surprisingly ethnic origin of the patients has a major influence on the likelihood to find a matched donor because of the underrepresentation of "non-Caucasian" donors in the international registry. For example, based on the NMDP data, "Asian" patients have a two-fold higher probability to have a mismatched donor compared to "Caucasian" patients [19]. In a single center the donor (7-8/8 match) identification rate was about 90% for patients classified as "US or European Caucasians", 76% for "Hispanics", 62% for "Black/African American", and 33% for "Asians" [20].

In our experience we could identify a 10/10 or 9/10 matched donor in 71.2% patients in 2002–2005 (350 searches, mean 4.9 donors tested/patient) [6], and in 81.8% patients in 2010-2011 (274 searches, mean 5.1 donors tested/patient) (Table 4). In 2011 the average number of tested donors/patient was similar for all 3 categories (4 donors/patient), but lower than in 2010 (data not shown). The efficiency of searches run at the LNRH in 2010-2011 was evaluated by computing the time frame between the start of the search and the date of the HLA report providing the best matched donor, that is, a 10/10 matched donor for the high probability searches, or 9-10/10 matched donor for the low/intermediate searches, and with date of transplantation. For the high probability searches run in 2010-2011 that led to a transplant the average time to propose a donor to the transplant center was 54 days (Table 5). This is comparable to the 1.4 months median search time reported for Northwestern European patients [16] and the 1.7 months time reported for Austrian patients [21]. This duration was however longer than the 21-days mean time reported for successful searches run by the German study [13]. Considering the nontransplanted patients with a high probability estimate the time frame for donor identification was identical. For the intermediate probability searches the time frame was 73 (34–217) days, and for the low probability searches the time frame was 83 (33–308) days (data not shown). For these 2 categories the search time was therefore longer than the average time reported in other studies [13, 21], but comparable to the duration reported by the U.K. study of searches run in 2005 [17]. Interestingly the time to transplant was similar for high and low probability searches, but slightly lower for intermediate probability searches (Table 5).

6. Clinical and Functional Relevance of Single HLA Mismatches

Whereas there is a consensus on the negative impact of single mismatches at HLA-A, B, C, DRB1 loci, the most difficult issue in selecting a 9/10 matched donor concerns the nature of the accepted mismatch. HLA-DQB1 incompatibilities are usually more readily accepted [1–3, 22]. In the NMDP study [3] HLA-A and -DRB1 mismatches were reported to have a more detrimental impact on overall survival than HLA-B and -C mismatches. On the other hand a recent analysis of unrelated donor peripheral blood HSC transplants from NMDP reported that only HLA-C antigen and HLA-B allele or antigen mismatches were associated with mortality [23]. In the Japan Marrow Donor Program (JMDP) study, HLA-A/B mismatches, but not HLA-C/DRB1/DQB1, were found to be significantly associated with reduced overall survival [24]. HLA disparities might reveal a stronger negative impact in those patients that have less advanced disease [1–3, 25] or other risk factors. There are no conclusive data showing

a difference between allele-level and antigen-level mismatches [3, 26]. Furthermore, one should be careful in interpreting the permissivity of a given locus as identified in retrospective studies, because of possible bias in the accepted mismatches. For example, the role of DRB1 incompatibilities could be underestimated in patients study groups if a significant number of DRB1*11:01 versus *11:04 mismatched pairs are included. It is perhaps not a surprise that the negative impact of HLA-C mismatches is reported with a high statistical significance, as compared to A,B,DRB1 mismatches, since incompatibilities do occur more frequently at HLA-C locus and are often more readily accepted by the transplant centers. A hierarchy in the relevance of HLA incompatibilities must be considered in light of other patient/donor risk factors, as proven by the high predictive value of the EBMT risk score [27, 28]. *A fortiori* the ranking of individual permissive mismatches will be impossible to define unless extremely large patients cohorts can be analysed [29]. Some HLA incompatibilities have been shown to be potential permissive mismatches by *in vitro* cytotoxic T lymphocyte precursor (CTLp) frequency assays, as exemplified by the C*03:03 versus *03:04 disparity [30, 31].

7. Evaluation of HLA Mismatches at the Amino Acid Level

Other strategies for disclosing less detrimental mismatches have focused on the nature of the mismatch at the amino acid (aa) level. The HistoCheck scoring system for HLA class I mismatches, based on functional similarity of aa involved in antigenic peptides and T-cell receptor binding turned out not to be predictive of clinical outcome [32]. An evaluation of the impact of individual HLA mismatches, such as those reported in the JMDP study [33] may not be applicable in other populations which show a much larger heterogeneity in HLA disparities and therefore fewer mismatches of similar nature [29]. Using a novel statistical methodology, Marino et al. [34] have reported 13 aa substitutions associated with increased mortality at day 100 in low/intermediate risk patients transplanted with HSC from a single HLA class I mismatched donor. In a recent study [35], the alloreactive CTLp frequency determined in single HLA-A and -C incompatibilities was associated with the aa differences between the mismatched alleles. The probability of a negative CTLp was higher in pairs with >9 aa differences compared to pairs with 0–5 aa differences in the α-helices and β-sheet. Eight aa (62, 63, 73, 80, 116, 138, 144, 163) were most predictive for a negative CTLp frequency analysis. It is however difficult to compare this model with the random forest analysis mentioned above since 7 of the 12 aa substitutions associated with a negative CTLp outcome are reported to be associated with lower 100 day-survival in the NMDP analysis [34]. At least these models should be tested on independent patients cohorts. CD8+ T-cell alloreactivity, as determined by intracellular staining for IFN-γ, has been reported to be higher for HLA-B than for HLA-A mismatches [36]. This observation is not consistent with the more detrimental impact of HLA-A disparities reported in the NMDP study [3].

8. Conclusion

As evaluated in searches for patients mainly of European ancestry, a 9-10/10 HLA matched donor can be identified for 60–80% patients. Many transplant centers are now using search algorithms based on allele/haplotype frequencies in order to take earlier decisions to transplant with a mismatched donor or to select an alternative donor (e.g., cord blood, haplo-identical donor) or a nontransplant strategy. In our preliminary experience, the inclusion of prospective HLA-DPB1 typing in the search algorithm for those patients with more than one 10/10 allele matched donor has allowed to identify a 12/12 matched donor for about 40% patients. The challenge remains to reliably predict the functional relevance of individual mismatches for low probability searches, but at least some models are testable. Considering the multiple clinical variables in HSCT, as represented partially by the EBMT risk score [27], it is likely that only clinical studies with more homogenous patients cohorts will be informative. Parameters such as urgency of the transplantation, T-cell depletion, and reduced intensity conditioning might well impact on the role of HLA disparities. At the present time the ranking of HLA-A, B, C, or DRB1 mismatches still appears elusive. We consider the possibility that an *in vitro* functional assay may be used in the algorithm provided it is simple enough, requires limited amount of blood, and is quantitatively highly reproducible. MHC-linked non-HLA genetic polymorphisms that do impact clinical outcome [5, 37, 38] could also be included in the algorithm, primarily for the high probability searches, if validated by larger scale studies.

Acknowledgments

The author is grateful to L. Quiquerez, M. Bujan, and the technicians of the LNRH, and to Dr. G. Nicoloso from Swiss Blood Stem Cells for their expert collaboration. He also acknowledges the most efficient collaboration with Professor J. Passweg, Professor Y. Chalandon, Dr. U. Schanz, and Dr. T. Güngör, heads of the four Swiss centers for allogenic HSCT. This work has been supported by Grant 320030_130483 from the Swiss National Science Foundation.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 271695, 6 pages
doi:10.1155/2012/271695

Review Article

Role of Killer Immunoglobulin-Like Receptor and Ligand Matching in Donor Selection

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Received 16 August 2012; Accepted 1 October 2012

Academic Editor: Andrzej Lange

Despite all efforts to improve HLA typing and immunosuppression, it is still impossible to prevent severe graft versus host disease (GVHD) which can be fatal. GVHD is not always associated with graft versus malignancy and can prevent stem cell transplantation from reaching its goals. Overall T-cell alloreactivity is not the sole mechanism modulating the immune defense. Innate immune system has its own antigens, ligands, and mediators. The bridge between HLA and natural killer (NK) cell-mediated reactions is becoming better understood in the context of stem cell transplantation. Killer immunoglobulin-like receptors (KIRs) constitute a wide range of alleles/antigens segregated independently from the HLA alleles and classified into two major haplotypes which imprints the person's ability to suppress or to amplify T-cell alloreactivity. This paper will summarize the impact of both activating and inhibitory KIRs and their ligands on stem cell transplantation outcome. The ultimate goal is to develop algorithms based on KIR profiles to select donors with maximum antileukemic and minimum antihost effects.

1. Introduction

Allogeneic hematopoietic stem cell transplantation is a curative approach. Removal of residual malignant cells and relapse prevention by an intensive conditioning regimen and reinstitution of a successful posttransplant anticancer immune response are the essential benefits of this treatment modality. The current donor-recipient matching criteria involve multiple factors but the only immunological barrier taken into consideration is the human leukocyte antigen system (HLA). However, an important factor affecting the success is the function of natural killer (NK) cells which are closely controlled by KIRs that interact with specific HLA class I ligands. KIR genes are encoded within 100–200 kb region of the leukocyte receptor complex (LRC) located on chromosome 19 (19q13.4) and segregated independently from the HLA genes. Most of HLA identical donor-recipient matched pairs are actually KIR mismatched. Innate system involves natural killer cells which through binding to their ligands can inhibit or activate the anticancer or antidonor reactivity arising from HLA recognition. The KIR genes belong to the most polymorphic structures between all surface receptors, second only to MHC, and are the key regulators of NK cells. Since these receptors are located on natural killer cells, they are called killer immunoglobulin-like receptors (KIRs). KIRs may exert inhibitory or activating functions through iKIR and aKIRs. There are nine iKIR and six aKIR receptors. The number of Ig-like domains on their extracellular region and the length of the cytoplasmic tail of the KIR proteins define the acronym for each KIR gene. Most of them have 2 Ig-like domains D1, D2 or D0, D2 (KIR2D), and the others have 3 domains D0, D1, and D2 (KIR3D). Receptor families with a long tail, "L" KIRs, are mostly inhibitory (e.g., KIR2DL, KIR3DL); whereas short tail ones, "S" KIRs, are mostly activating (e.g., KIR2DS, KIR3DS) with an exception, KIR2DL4, which has a potential for activating or inhibitory function. Some but not all of natural killer receptor ligands have been defined. Some are HLA class I

molecules including HLA-A (A3, A11) for KIR3DL2, HLA-B (Bw4) for KIR3DL1, HLA-C^{Lys80}, C^{Asn80}, for KIR2DL1 and KIR2DL2/3, respectively, and HLA-G for KIR2DL4 antigen subgroups. The HLA-C ligands are grouped according to their residue on position 80. The acronym of group 1 HLA-C is C1 or C^{Lys80} (HLA*C 01, 03, 07, 08, 12, 13, 14, and 16 and B*4601, B*7301) and group 2 HLA-C is C2 or C^{Asn80} (HLA*C 02, 04, 05, 06, 15, 17, and 18) [1, 2].

KIR diversity among people may originate from three reasons: allelic variations, the level of expression on the cell surface, and the haplotypic variability. Based on population studies KIR alleles are organized into two broad haplotypes: haplotype A and B. Haplotype A constitutes of 7 KIR genes, 6 inhibitory KIRs including the 4 framework genes plus the only activating gene KIR2DS4. Haplotype B is characterized by the presence of 1–5 activating KIR genes beside the increased number of genes with a greater variability, generated from recombinations of a centromeric and a telomeric cluster. Homozygosity of Haplotype A versus B defines an individual's ability to amplify or suppress immune reactions. Since NK cells can recognize donor antigens from tumor antigens, at least normal NK cell reactivity is essential for a graft versus leukemia effect in the absence of graft versus host reaction. However, to complicate the events further, even in the presence of activating KIR genes, these reactions can be silenced leading to abolition of activity.

Many investigators have evaluated the role of KIR receptor polymorphisms, KIR receptor-ligand matching on transplant outcome. The variation among studies in regard to donor or stem cell types, conditioning regimens, use of T-cell depletion has demonstrated a complex picture. To complicate analysis even further, factors that increase relapse or GVHD rates, such as disease activity at transplantation or gender matching, are not always similar between these studies. It is hypothesized that KIR-ligand mismatching is prerequisite for NK alloreactivity and, thus HLA mismatched transplants exert the best models for studying innate immune system activities [1, 2].

Previous reviews have grouped these NK alloreactivity studies in four models:

(1) KIR-ligand incompatibility, or ligand-ligand model (Ruggeri et al.);

(2) receptor-ligand model (Leung et al.);

(3) KIR gene-gene (receptor-receptor or haplotype) model (initially described by Nantes group, actually is similar to the Stanford model) (Gagne et al., Parham et al., and McQueen et al.);

(4) missing ligand model (retrospective model actually similar to the receptor-ligand model but neither the donor KIR nor HLA is considered for donor selection).

In summary, except for the fourth model which is a retrospective evaluation, the other models are based on biological matching principles and are being used for donor selection [1–5].

In this paper, these reports will be categorized and summarized according to stem cell source, donor matching, and conditioning regimens (Tables 1 and 2). It is an attempt towards a guide for use of KIR allele-ligand matching in donor selection.

2. KIR Matching in Haploidentical Stem Cell Transplants

Although NK cells activity against malignant cell were known for a long time, it was only in late 90s that the impact of KIR-ligands on allogeneic transplant was investigated. Haploidentical transplants have been an ideal model to investigate these effects. Following the initial reports by Valiante and Parham, the Perugia group and later additional groups investigated the impact of donor-ligand matching status after T cell depleted haploidentical transplantation for AML [3, 6–8]. In the presence of KIR-ligand mismatch between donor-recipient pairs, improved engraftment and a decrease in relapse rates were observed [1–3]. These effects were restricted to patients transplanted only in CR. However, subsequent studies were not able to confirm these results, directing investigators to analyze additional parameters. Recently, a study on haploidentical transplants with a posttransplant cyclophosphamide infusion have confirmed the role of haplotype B to have a GVL effect and prolongation of survival similar to the results obtained between siblings or matched unrelated subjects [7]. Multiple factors such as high T-cell content of the graft, suboptimal dose of T-cell depletion and HLA_mismatch level may effect NK-cell reconstitution and mask KIR effects [2]. A recent publication supports the following statement: T-cell alloreactivity overrides NK-mediated responses and optimal immunosuppression liberates NK-cell effects against leukemia. In other words, if extensive T-cell depletion such as CD34+ selection is performed, such as the setting of haploidentical transplantation, KIR-ligand mismatching benefits become visible [2, 3, 6–8].

3. KIR Gene-Gene Matching in Sibling Identical Stem Cell Transplants

As seen in Tables 1 and 2, Hsu, McQueen, Verheyden, Kim, Dalva, and Stringaris published reports analyzing the impact of KIRs utilizing KIR genotype, KIR haplotype, or telomeric KIR haplotype matching models [5, 10–14]. Four of these studies showed a beneficial effect of the B haplotype,which contains more activating KIRs, on both survival and relapse. These results are in accordance with results observed following haploidentical transplants by Ruggeri et al. [3] and Symons et al. [7]. It is important to note that both of the inconsistent studies include in vivo T depletion which might have unleashed NK-cell alloreactivities. However even the results from these two studies are not similar.

4. KIR Matching in Unrelated Myeloablative Stem Cell Transplants among Adults

Donor KIR3DS1, which is an activating KIR and is part of the haplotype B, is observed among 33% of donors. Transplants

Table 1: Characteristics of studies analyzing KIRs or KIR ligands.

Reference	*n*	Stem cell source	T-cell depletion	Donor type	HLA match	Diagnose
Ruggeri et al. 2002 [3]	92	PB	All	Related	Haploidentical	Various
Bishara et al. 2004 [6]	62	PB	Not all	Related	Haploidentical	Various
Symons et al. 2010 [7]	86	BM	None	Related	Haploidentical	Various
Weisdorf et al. 2012 [8]	24	PB	All	Related	Haploidentical	Myeloid
Cook et al. 2004 [9]	220	?	?	Related	HLA match	Lymphoid, myeloid
Hsu et al. 2005 [10]	178	BM	All	Sibling	HLA match	Various
Dalva et al. 2006 [11]	84	PB/BM	None	Sibling	HLA match	Various
McQueen et al. 2007 [5]	202	PB (89%)/BM	None	Sibling	HLA match	Various
Kim et al. 2007 [12]	53	BM/PB	None	Sibling	HLA match	Myeloid
Giebel et al. 2009 [13]	100	PB/BM	All	Sibling/unrelated	HLA match (81%)	Various
Stringaris et al. 2010 [14]	246	PB/BM	All	Sibling	HLA match	Myeloid
Davies et al. 2002 [15]	175	BM	34%	Unrelated	HLA mismatch	Various
Giebel et al. 2003 [16]	130	BM (96%)	81%	Unrelated	HLA match (47%)	Various
Bornhäuser et al. 2004 [17]	118	BM/PB	All	Unrelated	HLA match (46%)	Myeloid
Schaffer et al. 2004 [18]	190	BM/PB	All	Unrelated	HLA match (49%)	Various
Beelen et al. 2005 [19]	374	BM/PB	None	Unrelated (60%)	HLA match (63%)	CML (63 %)
De Santis et al.2005 [20]	104	BM/PB	14% (BM)	Unrelated	HLA mismatch	Various
Kröger et al. 2005 [21]	73	PB (63%)/BM	All	Unrelated	HLA match (86%)	Myeloma
Farag et al. 2006 [22]	1571	BM	None	Unrelated	HLA match (64%)	Various
Miller et al. 2007 [23]	1770	PB/BM	None	Unrelated	HLA match	Various
Yabe et al. 2008 [24]	1489	BM	All	Unrelated	HLA match	Various
Cooley 2009 [25]	448	?	None	Unrelated	HLA match (47%)	Myeloid
Cooley et al. 2010 [26]	1086	?	None	Unrelated	HLA match (50%)	Myeloid, lymphoid
Gagne et al. 2009 [4]	264	BM	None	Unrelated	HLA match (62 %)	Various
Venstrom et al. 2010 [27]	1087	BM (97%)	19%	Unrelated	HLA match (62%)	Myeloid, lymphoid
Brunstein et al. 2009 [28]	257	CB	32%	Unrelated	HLA mismatch (92%)	Various
Willemze et al. 2009 [29]	218	CB	81%	Unrelated	HLA mismatch	Various

BM: bone marrow, PB: peripheral blood, CB: cord blood, and CML: chronic myeloid leukemia.

performed with stem cells from donors positive for 3DS1 led to a decrease in grade II-IV GVHD and TRM without increasing relapse rate. This effect was amplified among subjects who were homozygous for this phenotype [27]. These authors have also reported a similar GVHD protection effect of Bw4 that was amplified in the presence of 3DS1. The overall effect of haplotype B on GVHD was dependent on 3DS1 and the other aKIR, 2DS2. These aKIRs are in strong linkage disequilibrium. Thus it was concluded that donor KIR 3DS1 and Bw4 expression, additively protects recipients from GVHD and TRM, without hampering the GvL effect.

5. KIR Matching in Unrelated Cord Blood Transplants

Similar to haploidentical transplants, cord blood transplants also utilize highly mismatched donors allowing a prominent GVHD effect that can be investigated under the context of KIR matching [28, 29]. So far, there are two major reports with opposing results. As seen in Tables 1 and 2, the type of conditioning regimens, in vivo T depletion, and number of cord blood units were different between these studies leading to a detrimental effect of KIRs following reduced intensity conditioning regimen. Based on existing data, it is not possible to establish criteria for cord blood selection. There is certainly need for prospective studies analyzing the effect of KIRs and KIR-ligand matching in both GVH or HVG directions.

6. KIR Matching for Donor Selection

Finally, the first attempts of donor selection criteria based on KIR genotyping have been proposed: the studies by Cooley et al. demonstrated protection against relapse and survival benefit when donors with certain KIR B genotypes are used for T-cell replete unrelated donor HCT for AML suggesting KIR genotyping to be incorporated into unrelated donor selection algorithm [25, 26]. This finding is supported by data from sibling transplants, with the exception of data from Stanford and us, reporting an increase in relapse associated with haplotype B [5, 11, 12, 14]. On the contrary, Stringaris et al., also based on data from sibling transplants, have reported a positive effect of haplotype B on survival. Through three groups including us, we were able to show the presence of activating KIRs to augment graft versus host/leukemia immunity whereas the inhibitory KIRs cause

Table 2: Impact of KIR or KIR ligand matching on transplant outcome.

Reference	Overall survival	aGVHD	Graft failure	Relapse
Ruggeri et al. 2002 [3]	Better (missing KIR ligand)	Decrease (missing KIR ligand)	Decrease (missing KIR ligand)	Decrease (missing KIR ligand)
Bishara et al. 2004 [6]	Better (KIR match, GVH direction)	increase (donor aKIR)	No effect	No effect
Symons et al. 2010 [7]	Better (iKIR mm, D: haplotype B)	No effect	—	Decrease (iKIRmm; haplotype B: D/R: +/−) myeloid, lymphoid
Weisdorf et al. 2012 [8]	No effect	No effect	—	No effect (KIR increase ligand mm)
Cook et al. 2004 [9]	Unknown (haplotype A: CMV reactivation)	Unknown	—	Unknown
Hsu et al. 2005 [10]	Better (missing iKIR ligand)	No effect	—	Decrease (AML, MDS, and missing iKIR ligand)
Dalva et al. 2006 [11]	Better (aKIR m)	Decrease (iKIR m)	—	Decrease (aKIR m) Increase (D: haplotype B)
McQueen et al. 2007 [5]	Worse (donor but not recipient has haplotype B)	Increase (donor but not recipient has haplotype B, also Bw4)	—	Increase (haplotype B D/R: +/−) Decrease (D: 3DL1/3DL2; R: A3/11or Bw4+)
Kim et al. 2007 [12]	Better (D: aKIR)	Increase (D: aKIR: 2DS2-4)	—	Decrease (D: aKIR)
Giebel et al. 2009 [13]	Decrease (aKIR mm and group C2+)	Increase (aKIR mm)	—	Increase (aKIR mm)
Stringaris et al. 2010 [14]	Better (D: haplotype B)	Unknown	—	Decrease (D: aKIR or haplotype B) AML
Davies et al. 2002 [15]	Worse (missing KIR ligand) myeloid	No effect	No effect	No effect
Giebel et al. 2003 [16]	Better (KIR ligand mm)	No effect	Increase (KIR ligand match)	Decrease (KIR ligand mm) myeloid
Bornhäuser et al. 2004 [17]	No effect	No effect	—	Increase (KIR ligand mm)
Schaffer et al. 2004 [18]	Worse (increase infections)	No effect	—	No effect
Beelen et al. 2005 [19]	No effect	No effect	Increase (KIR ligand mm)	Decrease (KIR ligand mm)
De Santis et al. 2005 [20]	Worse (KIR epitope mm)	Increase (NK epitope mm)	Worse (NK epitope mm)	—
Kröger et al. 2005 [21]	No effect	Not significant	—	Decrease (KIR ligand mm)
Farag et al. 2006 [22]	No effect	No effect	No effect	No effect
Miller et al. 2007 [23]	—	Increase (KIR ligand mm)	—	Decrease (both KIR ligand and HLA mm)
Yabeet al. 2008 [24]	Worse (KIR ligand mm)	Increase (KIR ligand mm; D:2DS2)	—	No effect
Cooley et al. 2009 and 2010 [25, 26]	Better (D: haplotype B)	No effect	No effect	Decrease (D: haplotype B) AML but not ALL
Gagne 2009 [4]	No effect (D: haplotype B); Decrease (HLA identical, KIR3DL1: D+R− D: KIR3DL1+/3DS1+ R: Bw4+ R: C1 ligand−)	Increase (HLA I: 2DL5 mm HLA nonI: 2DS1mm) Decrease (HLA I: 2DS3 mm, D: haplotype B)	—	No effect (D: haplotype B) Increase (D: 3DL1+/3DS1+ R: Bw4−) Decrease (D: 3DL1+/3DS1+ R: Bw4+)
Venstrom et al. 2010 [27]	Better (D: KIR3DS1)	Decrease (D: KIR3DS1)	—	No effect (D: 3DS1)
Brunstein et al. 2009 [28]	Worse (only with RIC)	Increase (KIR ligand mm)(RIC)	—	Decrease (KIR ligand mm) (RIC)
Willemze et al. 2009 [29]	Better (KIR ligand mm)	Decrease	—	Decrease (KIR ligand mm)

M: match, mm: mismatch, RIC: reduced intensity conditioning, D/R: donor/recipient, HLA I: HLA identical, and HLA nonI: HLA nonidentical.

immune tolerance. This effect is observed frequently, if not exclusively, among patients with myeloid disorders [11, 12, 14]. In spite of all inconsistencies and contradictory results which are usually arising from the lack of simultaneous evaluation of donor and recipient KIR status; disease or conditioning regimen type related heterogeneity between studies, Leung was able to propose a donor selection Algorithm [2] as follows.

(I) More Than One HLA-Matched Donor Available (Sibling, Unrelated, or Cord Blood)

Selection of donor with receptor-ligand mismatch in KIR.

Selection of donor with "B" haplotype in KIR.

No need to consider KIR-ligand mismatch (as KIR-ligands always match if HLA matches).

(II) HLA-Matched Donor Not Available; T Cells Not Depleted (Related and Unrelated)

Selection of donor with the least degree of HLA mismatch.

Selection of donor with receptor-ligand mismatch in KIR.

Selection of donor with "B" haplotype in KIR.

Avoid donor with KIR-ligand mismatch.

(III) HLA-Matched Donor Not Available; T Cells Depleted or Single-Unit Cord Blood Transplant

Selection of donor with receptor-ligand mismatch in KIR.

Selection of donor with "B" haplotype in KIR.

Selection of donor with KIR-ligand mismatch.

7. Conclusion

It appears that different KIR parameters are valid for each donor-recipient pair based on the degree of HLA matching, T-cell depletion intensity, and the type of leukemia. The protective or opposite effects of haplotype B among unrelated or sibling transplants is a perfect example for inconsistent results. Thus the algorithm presented by Leung is open to further discussion.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 257086, 12 pages
doi:10.1155/2012/257086

Clinical Study

Occurrence and Impact of Minor Histocompatibility Antigens' Disparities on Outcomes of Hematopoietic Stem Cell Transplantation from HLA-Matched Sibling Donors

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Received 26 June 2012; Revised 11 September 2012; Accepted 25 September 2012

Academic Editor: Bronwen Shaw

We have examined the alleles of eleven minor histocompatibility antigens (MiHAs) and investigated the occurrence of immunogenic MiHA disparities in 62 recipients of allogeneic hematopoietic cell transplantation (allo-HCT) with myeloablative conditioning performed between 2000 and 2008 and in their HLA-matched sibling donors. Immunogenic MiHA mismatches were detected in 42 donor-recipient pairs: in 29% MiHA was mismatched in HVG direction, in another 29% in GVH direction; bidirectional MiHA disparity was detected in 10% and no MiHA mismatches in 32%. Patients with GVH-directed HY mismatches had lower both overall survival and disease-free survival at 3 years than patients with compatible HY; also higher incidence of both severe acute GvHD and extensive chronic GVHD was observed in patients with GVH-directed HY mismatch. On contrary, GVH-directed mismatches of autosomally encoded MiHAs had no negative effect on overall survival. Results of our study help to understand why posttransplant courses of allo-HCT from siblings may vary despite the complete high-resolution HLA matching of a donor and a recipient.

1. Introduction

The allogeneic hematopoietic cell transplantation (allo-HCT) still remains a curative treatment of many severe diseases, especially hematooncological malignancies. The successful donor search is one of the most important factors deciding about the feasibility of transplantation. It starts with search among the patient's siblings as the HLA-matched sibling donor is regarded as the optimal one. The odds ratio for HLA compatibility in siblings is 1 : 4. The probability of having a matched sibling donor by a particular patient is determined by the formula $1 - (0.75)^n$, where n equals the number of siblings. Despite the improved matching of donor-recipient pairs that was possible after the implementation of high-resolution methods of molecular HLA typing, the better outcomes of transplantations are still limited by high number of complications: graft versus host disease (GVHD), engraftment problems (lack or loss of engraftment), and relapse [1]. The long-term survival after allo-HCT is being estimated in the range of 40–70%. Failures are mainly due to infectious complications and GVHD (30–40% each), organ toxicity of chemotherapy (20%), and relapse (20–30%) [2].

HLA matching remains the most important factor influencing both donor selection and transplantation outcomes. However, research of the human genome revealed that

polymorphism of nucleotides in genes that are non-HLA related (e.g., NOD2/CARD15 or genes encoding cytokines: TNF-alpha, IL-10, IL-6, interferon gamma, IL-1, and TGF-beta) may also determine the individual immunological phenotype of donor-recipient pairs, thus influencing GVHD, infections, and overall survival [3]. Minor histocompatibility antigens (MiHAs) belong to immunogenetic non-HLA related factors encoded by polymorphic genes, which may differ between the recipient and the donor and thus they may have impact on transplant outcomes.

The impact of antigens independent from Major Histocompatibility complex on transplantation results was first observed by Counce et al. in 1950s [4]. They explored graft rejection in inbred mice, which had undergone the transplantation of skin cells and neoplasmatic cells. Genes which were not associated with MHC responsible for slower course of rejection were called weak histocompatibility genes [4, 5]. The first hypothesis concerning potential impact of MiHA on the outcome of BMT (bone marrow transplantation) was based on a case of a female recipient (with severe aplastic anemia) who received a transplant from her brother. Graft rejection after BMT was diagnosed and reactivity of cytotoxic T cells isolated from peripheral blood of recipient was directed to antigens present on donor's cells which were not associated with HLA [6].

Minor histocompatibility antigens are polymorphic peptides consisting of 9–12 amino acids. After binding to the antigen recognition site of either class I or class II HLA molecules present on a cell surface MiHAs can be recognized by T-lymphocytes. Thus the occurrence of MiHA depends on the presence of specific HLA antigens, which is called the MHC restriction. MiHAs are encoded by either autosomal chromosomes or by Y-chromosome [7–9]. Disparities of MiHA may result from polymorphism of amino acids, gene deletions [10], or from several intracellular mechanisms [11]. MiHA disparity may originate from a single or several amino-acid substitution in the part of MiHA peptide recognized by TCR (T-cell receptor), like in the case of HY and HA-1. Amino-acid polymorphism may be present in the region of MiHA that binds to HLA molecule, causing different expressions of peptide-HLA complex in the donor than in the recipient. Polymorphism may also pertain proteins responsible for intracellular processing of peptides, what leads to the presence or absence of peptides (e.g., HA-2 or HA-8) on cell's surface [12], or phosphoproteins (e.g., SP-110, MiHA discovered in 2006 by Warren et al.) [13].

Most MiHA possess only one immunogenic allele, which is sufficient to induce MiHA immunogenicity [12]. Up to date 18 autosomal and 10 Y-chromosome encoded MiHAs have been identified; those tested in our study are presented in Tables 1 and 2.

There are two patterns of MiHAs' tissue distribution: restricted and broad. Autosomal HA-3, HA-8, and most of MiHAs encoded by Y-chromosome are present in most tissues, including those crucial for GVHD: skin, intestines, and liver [11, 12]. Most of autosomal and 2 MiHAs encoded by Y-chromosome (B8/HY and B52/HY) appear only in hematologic cells including leukemic cells, dendritic cells, NK, and multiple myeloma cells [40]. Thanks to their restricted distribution all of them may be potentially exploited in immunotherapy. The other type of MiHAs' tissue distribution is their appearance on epithelial neoplasmatic cells, for example, HA-1 and ACC-1/ACC-2 [41, 42], although in normal conditions they are restricted only to hematopoietic cells and are not present on epithelial cells.

Detection of MiHA bases most often on genomic typing with PCR-SSP method. The assessment of detected immunogenic disparities is simplified by the online availability of Leiden University Medical Center's dbMinor database [43]. Disparities of immunogenic MiHA alleles between the donor and the recipient may trigger GVHD and HVG reactions, which may lead to graft rejection or to GVH/GVL reaction [44–46]. T-lymphocytes directed against recipient specific MiHAs were detected in patients with GVHD [47]. In the group of 92 recipients of allo-HCT from unrelated donors, a higher incidence of chronic GVHD was observed in those with HY disparity [48]. Many clinical trials confirm that disparities of autosomally encoded MiHAs (like HA-1, HA-2, and HA-8) may increase the incidence of GVHD [15, 17, 22], while others did not confirm such dependence [49]. Female recipients after transplantation from male donors may experience graft failure due to HVG reaction against HY antigens resulting in a worse survival [3]. MiHA present on recipient's neoplasmatic cells (HA-1, HA-2, HA-8, HB-1, and HY) may constitute the target of cytotoxic CD8+ T-lymphocytes crucial for GVL reaction [12, 50], leading to the decrease of relapse rate [51]. Use of cytotoxic T-lymphocytes recognizing selectively only MiHA present on neoplasmatic cells enables the separation of GVL effect from GVHD [52]. Such MiHAs can be used both in vivo for the production of vaccines enhancing GVL reaction and in vitro as a load to antigen presenting cells stimulating reactivity of cytotoxic T-cells [53]. HA-1 and HA-2 are the most intensively explored MiHAs in immunotherapy [12, 52–54].

The aim of this study was to determine MiHA alleles and genotypes enabling to detect their immunogenic disparities in sibling donor-recipient pairs and to explore their influence on the results of allo-HCT.

2. Material and Methods

2.1. Patients and Donors. 62 patients: 34 women and 28 men of median age 38 (range 14–59) years, who received allo-HCT from siblings in the Department of Hematology and Bone Marrow Transplantation, Medical University of Silesia, Katowice, Poland, in years 2000–2008, entered the study. The indication for transplantation was acute myeloid leukemia (45 pts), acute lymphoblastic leukemia (14 pts), chronic myeloid leukemia in chronic phase, myelodysplastic syndrome, and resistant non-Hodgkin's lymphoma (1 pt each). Donors were 30 women and 32 men of median age 35 (range 14–60) years. Median followup was 3 (0.04–10) years.

2.2. Transplantation Procedure. Conditioning treatment was myeloablative (CyTBI: cyclophosphamide + total body irradiation in 12 pts, BuCy: busulfan + cyclophosphamide in

Table 1: Autosomally encoded MiHA.

MiHA	Restriction	Identification	Clinical trials	Protein	Tissue distribution	Presence on cells
HA-1	HLA-A*02	Den Haan et al. 1998 [14]	Goulmy et al. 1996 [15] Tseng et al. 1999 [16] Gallardo et al. 2001 [17]	HA-1	Restricted	Hematopoietic cells Bronchial carcinomas Cervix carcinoma Breast carcinoma Prostate carcinoma
HA-1/B60	HLA-B*60	Mommaas et al. 2002 [18]	—	HA-1	Restricted	Hematopoietic cells
HA-2	HLA-A*02	Den Haan et al. 1995 [19]	Goulmy et al. 1996 [15]	Myosin 1G	Restricted	Hematopoietic cells
HA-3	HLA-A*01	Spierings et al. 2003 [20]	Tseng et al. 1999 [16]	Lymphoid blast crisis oncogene	Broad	Hematopoietic cells Keratinocytes Fibroblasts PTECs HUVECs Melanocytes
HA-8	HLA-A*02	Brickner et al. 2001 [21]	Akatsuka et al. 2003 [22] Pérez-García et al. 2005 [23]	KIAA0020	Broad	Hematopoietic cells Fibroblasts
HB-$1^{H/Y}$	HLA-B*44	Dolstra et al. 1999 [24]	—	Unknown	Restricted	B cell ALL, EBV-BLCLs
ACC-1	HLA-A*24	Akatsuka et al. 2003 [25]	Nishida et al. 2004 [26]	BCL2A1	Restricted	Hematopoietic cells
ACC-2	HLA-B*44	Akatsuka et al. 2003 [25]	—	BCL2A1	Restricted	Hematopoietic cells
SP110 (HwA-9)	HLA-A*03	Warren et al. 2006 [13]	—	SP110 intranuclear protein	Restricted	Hematopoietic cells IFN—gamma inducible
PANE1 (HwA-10)	HLA-A*03	Brickner et al. 2006 [27]	—	PANE1	Restricted	Lymphoid cells
UGT2B17/A29	HLA-A*29	Murata et al. 2003 [28]	—	UGT2B17	Restricted	Dendritic cells, B-cells, EBV-BLCLs
UGT2B17/B44	HLA-B*44	Terrakura et al. 2007 [29]		UGT2B17	Restricted	Dendritic cells, B-cells, EBV-BLCLs

33 pts), reduced intensity (TreoFlu: treosulfan + fludarabine in 2 pts, TreoCy: treosulfan + cyclophosphamide in 2 pts), or nonmyeloablative (BuFlu: busulfan + fludarabine in 2 pts). Cumulative doses of drugs used in conditioning were busulfan 16 or 8 mg/kg p.o., cyclophosphamide 120 mg/kg i.v., treosulfan 42 g/m^2 i.v., fludarabine 150 mg/m^2 i.v. TBI dose was 12 Gy. Bone marrow was the source of hematopoietic cells in 40 patients, G-CSF-stimulated peripheral blood in 10 and both (harvest of insufficient number of CD34+ cells from the bone marrow followed by peripheral collection) in 12 patients. Details of transplanted cells are presented in Table 3. Standard GVHD prophylaxis consisted of cyclosporine A in initial dose 3 mg/kg i.v. starting from day −1 with dose adjusted to its serum level and shifted to oral administration about day +20, methotrexate 15 mg/m^2 i.v. on day +1 and 10 mg/m^2 i.v. on days +3 and +6. Methylprednisolone at dose 2 mg/kg i.v. was the first line therapy of aGVHD symptoms. The criteria defined by Glucksberg were used for the grading of aGVHD; the diagnosis and severity of cGVHD were determined according to NIH (National Institutes of Health) criteria established in 2005 [55].

2.3. Methods. DNA of patients and siblings was isolated from peripheral blood in the Biomolecular Laboratory of the Department of Hematology and BMT, Medical University of Silesia. Alleles of 11 autosomal and Y-chromosome encoded MiHAs were analyzed with PCR-SSP method for each donor-recipient pair in the Immunogenetics and HLA Laboratory of the Regional Blood Center in Katowice with the use of Dynal AllSet+ Minor Histocompatibility Antigen Typing Kit, according to a methodology recommended by Leiden University Medical Center. Products obtained in PCR-SSP reaction were analyzed on agarose gel and each detected allele encoding MiHA was translated into a specific letter code. dbMinor database of LUMC was used to determine the number, direction, and tissue distribution of MiHA mismatches on the base of MiHA alleles and HLA antigens

Table 2: Y-chromosome encoded MiHA.

MiHA	Restriction	Identification	Clinical trials	Protein	Tissue distribution	Presence on cells
A1/HY	HLA-A*01	Pierce et al. 1999 [30]	—	USP9Y	Broad	Hematopoietic cells, fibroblasts
A2/HY	HLA-A*02	Meadows et al. 1997 [31]	Goulmy et al. 1996 [15]	SMCY	Broad	Hematopoietic cells, fibroblasts
A33/HY	HLA-A*33	Torikai et al. 2004 [32]	—	TMSB4Y	Broad	Hematopoietic cells
B7/HY	HLA-B*07	Wang et al. 1995 [33]	—	KDMSD	Broad	Hematopoietic cells
B8/HY	HLA-B*08	Warren et al. 2000 [34]	—	UTY	Restricted	Hematopoietic cells
B52/HY	HLA-B*52	Ivanov et al. 2005 [35]	—	RPS4Y1	Restricted	Leukocytes, PHA blasts, EBV-BLCLs, B cells, breast carcinoma, hepatocellular carcinoma, colon adenocarcinoma, AML, ALL multiple myeloma
B60/HY	HLA-B*60	Vogt et al. 2000 [36]	—	UTY	Broad	Hematopoietic cells, fibroblasts
DRB1*1501/HY	HLA-DRB1*15	Zorn et al. 2004 [37]	—	DDX3Y (DBY)	Broad	Hematopoietic cells, fibroblasts
DRB3*0301/HY	HLA-DRB3*0301	Spierings et al.2003 [38]	—	RPS4Y1	Broad	Hematopoietic cells, fibroblasts
DQ5/HY	HLA-DQB1*05	Vogt et al. 2002 [39]	—	DDX3Y (DBY)	Broad	Hematopoietic cells, fibroblasts

Abbreviations: HUVE: human umbilical vein epithelium, PTE: proximal tubular epithelium, EBV-BLCL: Epstein Barr virus transformed B-lymphoblastoid cell lines, and PHA: phytohemagglutinine.

Data in Tables 1 and 2 are based on dbMinor database and materials presented during Minor Histocompatibility Workshop 2005, Leiden University Medical Center; Eric Spierings: minor H antigens: targets for tumor therapy—lecture at the conference "Immunogenetics in hematology and stem cell transplantation", Wroclaw 09.02.2006 and [8].

of respective donor-recipient pairs. The study has been approved by the responsible Ethical Committee of Medical University of Silesia.

2.4. Statistical Methods. Median, minimal, and maximum values were used to show numeric parameters of donor-recipient groups. Statistical analysis of MiHA mismatches' impact on transplantation outcomes was conducted in accordance to recommendation of EBMT [56]. MiHA mismatches were grouped according to mismatch direction (GVH or HVG), tissue distribution (restricted or broad), and the way of coding (autosomal or by Y-chromosome) in search for their influence on transplant results. Analysed endpoints included overall survival (OS), disease-free survival (DFS), aGVHD, and limited and extensive cGVHD. Kaplan-Meier method was used to estimate the probability of impact of MiHA mismatches on overall survival and disease-free survival. Results were presented as percent ±95% confidence interval (CI). The cumulative incidence method was used to evaluate the probability of relapse and GVHD (acute or chronic) in order to account events which may influence the outcome as a competing risk. Results were presented also in percent ±95% CI. Results with significance level $P < 0.05$ were considered statistically significant.

3. Results

3.1. Occurrence of Alleles and Genotypes and Their Mismatches. Immunogenic MiHA mismatches were detected in 42 (68%) donor-recipient pairs; 20 (32%) pairs had no mismatched MiHAs. Unidirectional HVG-directed disparities were observed in 18 (29%) pairs (in 9 pairs MiHA mismatches were encoded by Y-chromosome, in 8 pairs autosomally, and in 1 pair both autosomally and by Y-chromosome) and GVH-directed MiHA disparities were observed in another 18 (29%) pairs (in 9 pairs MiHA mismatches were Y-chromosome encoded, in 7 pairs autosomally, and in 2 pairs both autosomally and Y-chromosome

Table 3: Patients characteristics ($n = 62$).

	Median (range)	Quartiles
Age (years)		
Donor	35 (14–60)	26–49
Recipient	38 (14–59)	28–47
Time from diagnosis to allo-HCT (years)	0.62 (0.24–12.91)	0.5–1.12
	n	%
Sex		
Donor		
Male	32	51.6
Female	30	48.4
Recipient		
Male	28	45.2
Female	34	54.8
Donor/recipient		
Male/male	16	25.8
Female/female	18	29
Male/female	16	25.8
Female/male	12	19.4
Compatibility of ABO blood groups		
Compatible	36	58.1
Minor incompatibility	8	12.9
Major incompatibility	14	22.5
Minor and major incompatibility	4	6.5
Diagnosis		
AML	45	72.5
ALL	14	22.5
CML	1	1.61
MDS	1	1.61
NHL	1	1.61
Regimen		
TBI + cyclophosphamide	12	19.35
Chemotherapy		
Busulfan + cyclophosphamide	33	53.2
Treosulfan + fludarabine	13	20.96
Busulfan + fludarabine	2	3.22
Treosulfan + cyclophosphamide	2	3.22
Source of hematopoietic cells		
Bone marrow	40	64.5
Peripheral blood	10	16.1
Bone marrow and peripheral blood	12	19.4
	Median (range)	Quartiles
Number of transplanted cells		
Nucleated cells (NC) $\times$ 10*e*8/kg	3.51 (0.12–72.15)	2.34–5.84
CD34(+) $\times$ 10*e*6/kg	2.77 (0.95–10.50)	1.68–4.19
CD3(+) $\times$ 10*e*7/kg	3.84 (0.20–46.90)	2.71–18.01
Time range of allo-HCT	01.2000–12.2008	

Table 4: The occurrence of MiHA mismatches in GVH and HVG direction in 62 related donor-recipient pairs.

Immunogenic MiHA mismatches	In GVH direction	
	Present	Absent
In HVG direction		
Present	10% (6 pairs)	29% (18 pairs)
Absent	29% (18 pairs)	32% (20 pairs)

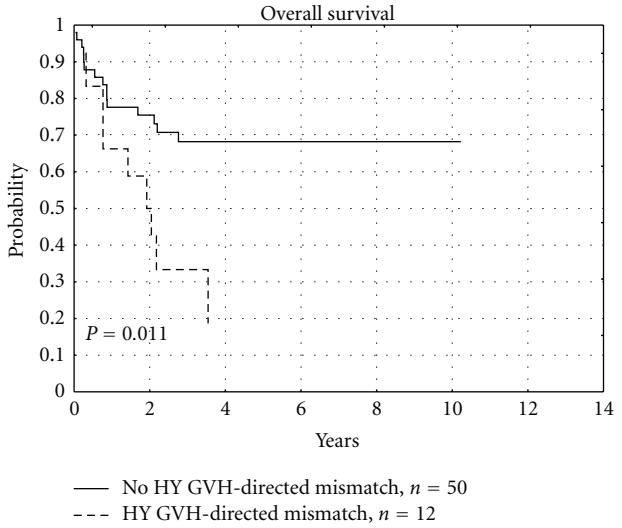

Figure 1: Influence of Y-chromosome encoded GVH-directed MiHA mismatch on overall survival.

encoded). In 6 (10%) pairs bi-directional (both HVG and GVH in the same donor-recipient pairs) MiHA mismatches were observed. The direction of MiHA mismatches is presented in Table 4 and the distribution of 11 MiHA alleles and genotypes in 62 related donor-recipient pairs is presented in Tables 5 and 6.

3.2. Impact of Immunogenic MiHA Mismatches on Allo-HCT Outcomes. Analysis of overall survival showed unfavorable impact of GVH-directed Y-chromosome encoded MiHA mismatches ($P = 0.011$), as presented in Figure 1 and Table 7, and favorable trend in case of GVH-directed autosomal MiHA disparities ($P = 0.045$), as presented in Figure 2 and Table 7.

GVH-directed mismatches of Y-chromosome encoded MiHA influenced unfavorable the disease free-survival ($P = 0.05$), as shown in Figure 3 and Table 7.

Serious (grade III or IV) acute GVHD was observed in 24 patients and it was influenced by Y-chromosome encoded GVH-directed MiHA mismatches ($P = 0.037$), which is presented in Figure 4 and Table 7.

The tissue distribution of GVH- or HVG-directed MiHA mismatches did not influence the incidence of aGVHD, neither grades I-IV, nor II-IV. Higher probability of extensive chronic GVHD was observed when Y-chromosome encoded GVH-directed MiHA mismatches were present ($P = 0.017$, as shown in Figure 5 and Table 7).

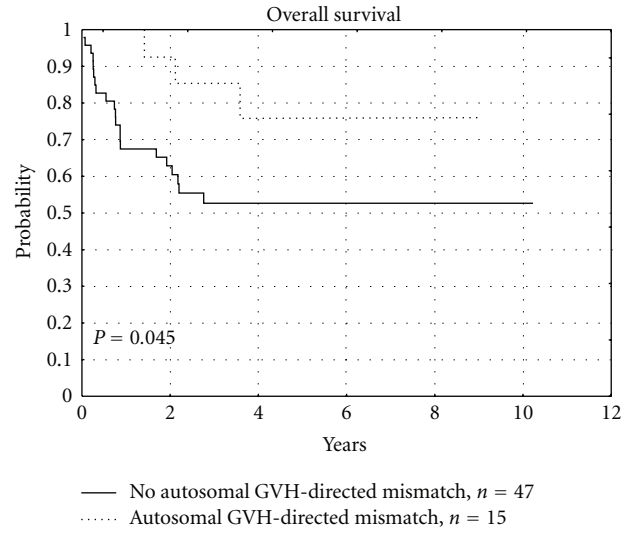

Figure 2: Influence of autosomal GVH-directed MiHA mismatch on overall survival.

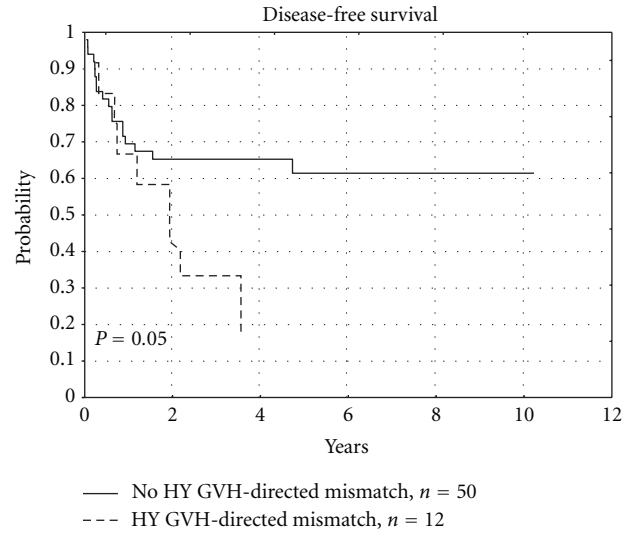

Figure 3: Influence of Y-chromosome encoded GVH-directed MiHA mismatch on disease-free survival.

The relapse following allo-HCT was observed in 15(24.2%) patients. Lower risk of relapse was observed in patients with HVG-directed MiHA mismatches: both autosomal (0.28(0.18–0.44) versus 0(0-0), $P = 0.032$) and with "restricted" pattern of tissue distribution (0.29(0.18–0.45) versus 0(0-0), $P = 0.028$). These data are presented in Table 7.

4. Discussion

Minor histocompatibility antigens belong to genetic factors which may vary between the donor and the recipient despite identical HLA and thus they may influence allo-HCT results. Knowledge of MiHA alleles and genotypes

Table 5: Distribution of 11 MiHA alleles in 62 related donor-recipient pairs.

MiHA	Allele	Recipient	Donor
HA-1	H	38.5%	41.8%
	R	61.5%	58.2%
HA-2	V	78.7%	73.0%
	M	21.3%	27.0%
HA-3	T	68.0%	70.5%
	M	32.0%	29.5%
HA-8	R	45.9%	45.9%
	P	54.1%	54.1%
HB-1	H	62.3%	64.8%
	Y	37.7%	35.2%
ACC-1	Y	23.0%	20.5%
	C	77.0%	79.5%
ACC-2	D	20.5%	19.7%
	G	79.5%	80.3%
SP110 (HwA9)	R	58.2%	58.2%
	G	41.8%	41.8%
PANE1 (HwA10)	R	67.2%	68.9%
	*	32.8%	31.1%
UGT2B17	+	86.9%	90.2%
	−	13.1%	9.8%
HY	+	50.8%	54.1%
	−	49.2%	45.9%

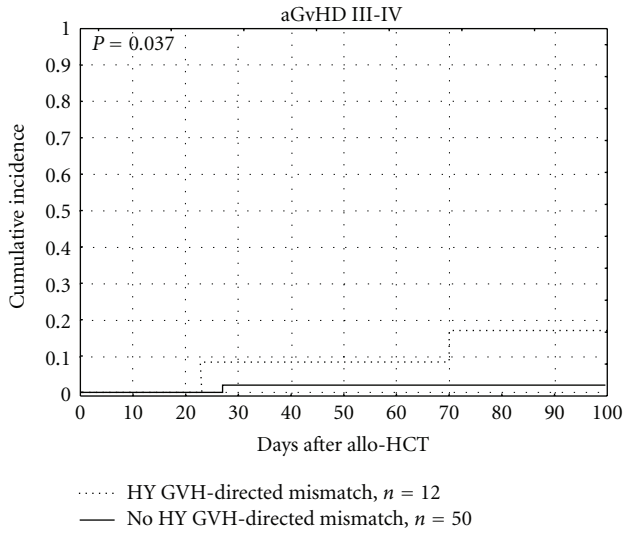

Figure 4: Influence of Y-chromosome encoded GVH-directed MiHA mismatches on serious aGVHD.

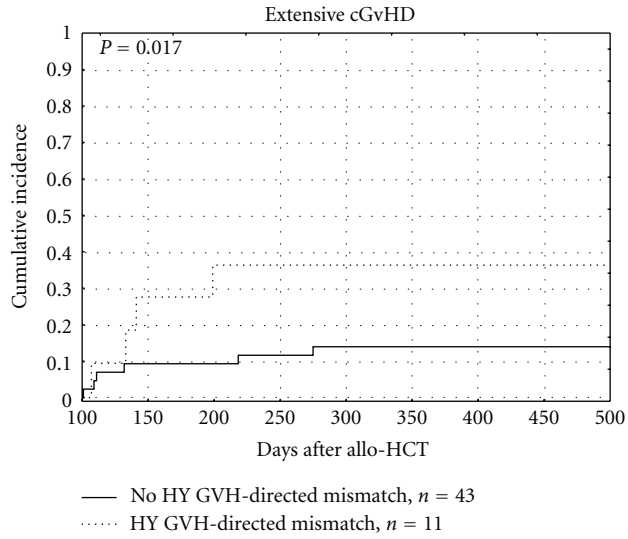

Figure 5: Influence of Y-chromosome encoded GVH-directed MiHA mismatches on extensive cGVHD.

enables to detect their disparities, which could be helpful not only in optimal matching of a donor/recipient pair and in understanding transplant results, but also it may create a chance to the use of MiHA in immunotherapy aiming to improve patients' survival [52–54]. The largest meta-analysis of MiHA distribution was performed by Spierings et al. who described the results of a multicenter trial of 10 MiHA distribution in 5 different ethnic groups worldwide. The study revealed significant differences in the frequency of MiHA alleles in dependence of geographical location, with UGT2B17 being the most variable MiHA [57]. Two MiHA trials have been performed in Polish population till now: in the first one HA-1 was analyzed in a group of 30 sibling pairs [58], another trial concerned the group of 92 unrelated pairs

Table 6: Distribution of MiHA genotypes' frequencies in 62 related donor-recipient pairs.

MiHA	Genotype	Recipient	Donor
	HH	13.1%	16.4%
HA-1	HR	50.8%	50.8%
	RR	36.1%	32.8%
	VV	59.0%	50.8%
HA-2	VM	39.3%	44.3%
	MM	1.6%	4.9%
	TT	44.3%	47.5%
HA-3	TM	47.5%	45.9%
	MM	8.2%	6.6%
	RR	27.9%	27.9%
HA-8	RP	36.1%	36.1%
	PP	36.1%	36.1%
	HH	34.4%	36.1%
HB-1	HY	55.7%	57.4%
	YY	9.8%	6.6%
	YY	4.9%	1.6%
ACC-1	YC	36.1%	37.7%
	CC	59.0%	60.7%
	DD	3.3%	0.0%
ACC-2	DG	34.4%	39.3%
	GG	62.3%	60.7%
	RR	27.9%	31.1%
SP110 (HwA9)	RG	60.7%	54.1%
	GG	11.5%	14.8%
	RR	42.6%	42.6%
PANE1 (HwA10)	R*	49.2%	52.5%
	**	8.2%	4.9%

++ or +− genotypes' frequencies of UGT2B17 and HY are equal to the frequency of alleles + and their −− genotypes' frequencies are equal to the frequency of alleles − presented in Table 5.

[12]. In our current study alleles and genotypes of 11 MiHAs have been estimated in 62 sibling donor-recipient pairs. Basing on our results and several other studies estimating the occurrence of specific MiHA mismatches in allo-HCT [59, 60], HA-1 can be regarded as a candidate target for immunotherapeutic applications.

We have observed the unfavorable impact of GVH-directed mismatches of Y-chromosome encoded MiHAs on OS ($P = 0.011$) and DFS ($P = 0.05$). Y-chromosome encoded MiHA represents MiHA with "broad" tissue distribution. Attack of donor's T-lymphocytes on recipients' tissues precipitated by HY mismatch could explain the increased occurrence of severe forms of acute and chronic GVHD, leading to earlier deaths of recipients. In our study recipients of allo-HCT from siblings did not receive anti-thymocyte globulin, what probably influenced the worse course, including the fatal course of their GVHD. We have shown that GVH-directed mismatches of HY correlate significantly with serious (III or IV) aGVHD and extensive cGVHD. These results correspond to the reported influence of sex difference on transplant outcomes, especially in the case of female donor to male recipient (FDMR) transplants [61, 62]. Oppositely, Markiewicz et al. in a study of 92 unrelated donor-recipient pairs found that HY mismatches in GVH direction influenced more favorable GVL effect than unfavorable GVHD, what resulted in the increased DFS ($P = 0.05$) [12, 63]. The probable explanation of this difference in MiHAs impact on OS and DFS between related and unrelated allo-HCT may be the use of stronger standard immunosuppressive prophylaxis including pretransplant antithymocyte globulin in unrelated allo-HCT setting. Increased incidence of serious acute and extensive chronic GVHD associated with mismatches of Y-chromosome encoded MiHAs, leading to a worse overall survival, may justify the administration of anti-thymocyte globuline before allo-HCT from sibling female donor to male recipient. Such approach could probably reduce the risk of GVHD originating from GVH-directed HY mismatch.

The analysis of GVH-directed mismatches of autosomal MiHAs, oppositely to HY, showed favorable trend to increase the OS, which was 76% in a mismatched versus 53% in a compatible groups at a 4-year posttransplant. Unlike GVH-directed HY disparities, those of autosomal MiHAs did not increase the occurrence of serious GVHD in our study, which contributed to the better survival. There are reports

Table 7: Influence of MiHA mismatches on allo-HCT outcomes.

Analyzed outcome	Analyzed MiHA	Direction of mismatch	Presence of mismatch	n	Probability (95% CI)	P
Overall survival	Autosomal	GVH	Yes	15	2 yrs: 0.9286 (0.5278–0.9892) 4 yrs: 0.7619 (0.3481–0.9130)	0.045
			No	47	2 yrs: 0.6046 (0.4329–0.7243) 4 yrs: 0.5265 (0.3511–0.6545)	
	HY	GVH	Yes	12	2 years: 0.4167 (0.0590–0.6384) 3 years: 0.3333 (0.0054–0.5532)	0.011
			No	50	2 years: 0.7546 (0.5986–0.8500) 3 years: 0.6822 (0.5152–0.7916)	
Disease-free survival	HY	GVH	Yes	12	2 years: 0.4167 (0.0590–0.6384) 3 years: 0.3333 (0.0054–0.5532)	0.050
			No	50	2 years: 0.6526 (0.4896–0.7635) 3 years: 0.6526 (0.4896–0.7635)	
Serious aGVHD	HY	GVH	Yes	12	0.1667 (0.0470–0.5906)	0.037
			No	50	0.0200 (0.0029–0.1392)	
Extensive cGVHD	HY	GVH	Yes	11	0.3636 (0.1664–0.7947)	0.017
			No	43	0.1395 (0.0664–0.2931)	
Relapse	Autosomal	HVG	Yes	12	0 (0-0)	0.032
			No	50	0.2836 (0.1818–0.4423)	
	Restricted	HVG	Yes	13	0 (0-0)	0.028
			No	49	0.2879 (0.1849-0.4482)	

describing the role of autosomal MiHAs in GVHD: for example, higher risk of aGVHD in the case of autosomal HA-1 incompatibility was reported in Tunisian group of 60 sibling donor-recipient pairs [64]. Others described increased incidence of cGVHD in the case of mismatched autosomal MiHAs localized on hematopoietic cells: HA-1, HA-2, and HA-8 [15, 16, 23, 65]. There are also reports that report no impact of autosomal MiHAs on GVHD [49, 66].

One could expect that disparities of MiHAs with broad tissue distribution present in the host should precipitate the posttransplant reaction of donor's lymphocytes and induce the GVHD. Unexpectedly, the tissue distribution of neither GVH- nor HVG-directed MiHA mismatches did not influence the incidence of GVHD.

Much lower probability of relapse following allo-HCT was observed by us in patients with HVG-, but not with GVH-directed MiHA mismatches. This finding, although intriguing, needs further confirmation as we do not find a reasonable explanation for this result. Japanese group found that GVH-directed HA-1 mismatches were associated with lower risk of relapse [51]. Similarly, experience of Polish group studying MiHAs in unrelated allo-HCT showed seldom episodes of relapse occurring when GVH-directed HY mismatches were present [63].

Results of our study help to explain why posttransplant courses of allo-HCT from siblings may vary despite complete high-resolution HLA-match and why cells interactions between the donor and the recipient may lead to serious complications.

5. Conclusions

GVH-directed HY mismatch significantly increased the occurrence of serious acute GVHD and extensive chronic GVHD and finally caused decreased overall survival. GVH-directed mismatches of autosomally encoded MiHAs had no negative effect on overall survival, which in fact was even longer. Findings of our study help to explain why the occurrence of immunological complications and in consequence final results of allo-HCT from high-resolution HLA-matched sibling donors are variable.

Conflict of Interests

The authors report having no conflict of interests.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 175419, 7 pages
doi:10.1155/2012/175419

Research Article

Computer Algorithms in the Search for Unrelated Stem Cell Donors

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Received 27 July 2012; Accepted 3 October 2012

Academic Editor: Colette Raffoux

Hematopoietic stem cell transplantation (HSCT) is a medical procedure in the field of hematology and oncology, most often performed for patients with certain cancers of the blood or bone marrow. A lot of patients have no suitable HLA-matched donor within their family, so physicians must activate a "donor search process" by interacting with national and international donor registries who will search their databases for adult unrelated donors or cord blood units (CBU). Information and communication technologies play a key role in the donor search process in donor registries both nationally and internationaly. One of the major challenges for donor registry computer systems is the development of a reliable search algorithm. This work discusses the top-down design of such algorithms and current practice. Based on our experience with systems used by several stem cell donor registries, we highlight typical pitfalls in the implementation of an algorithm and underlying data structure.

1. Introduction

Hematopoietic stem cell transplantation (HSCT) [1] (commonly referred to as bone marrow transplantation) is a medical procedure in the field of hematology and oncology, most often performed for patients with certain cancers of the blood or bone marrow. HSCT is the treatment of choice for people with hematopoietic malignancies, bone marrow failure, and certain types of cancer (e.g., lymphoma) which results in a compromised immune system. The most important factor in the successful outcome of HSCT is that the patient and donor are matched for the Human Leukocyte Antigens (HLA). The level of the matching required varies with the source of stem cells used for HSCT.

A lot of patients have no suitable HLA-matched donor within their family, so physicians must activate a "donor search process" by interacting with national and international donor registries who will search their databases for adult unrelated donors (AUD) or cord blood units (CBU).

Information and communication technologies play a key role in the donor search process in donor registries both nationally and internationaly. One of the major challenges for donor registry computer systems is the development of a reliable search algorithm. This work discusses the top-down design of such algorithms and current practice. Based on our experience with systems used by several stem cell donor registries, we will highlight typical pitfalls in the implementation of an algorithm and underlying data structure.

2. Search Algorithm

The purpose of the donor search algorithm is to find and present a selected list of potential donors and/or CBUs, in which those most likely to be an optimal stem cell source for the patient are sorted to the top of the list [2]. Selection and sorting criteria are based on HLA compatibility and may also take into consideration secondary preference criteria, such as CMV antibody status, gender, and age.

Basic requirements for the search system used by stem cell donor registries are as follow.

(i) *Deterministic*: behavior that ensures the same results with the same input. This means, the algorithm has to reproduce exact decisions at every step.

(ii) *Clear ranking order*: results.

(iii) *Exhaustive*: all donors available for transplant in the source database should be included in the search

algorithm. Exceptions must be clearly indicated to the end-user. For example, some algorithms exclude donors that are typed only at HLA-A and HLA-B.

(iv) *Scalable*: the system should be able to handle databases of varying size and type.

(v) *Fast*: search algorithms are also used in user-interactive systems, so the results should be received in seconds.

(vi) *Configurable*: search coordinator must be able to define patient-donor HLA match criteria and secondary preference criteria (CMV status, gender, and age).

(vii) *Consistently matched*: The data presented should be uniformly matched as a set for a given instance of a patient search. Different primary algorithms or matching criteria shall not be used within a single patient search.

The search algorithm is usually implemented as the key component of the stem cell donor registry software system. It has several inputs and a single output (see Figure 1). The following input data are essential.

(i) Patient's data: HLA type (minimum HLA-A, HLA-B, and HLA-DRB1 typing).

(ii) Patient's match criteria (position and number of allowable mismatches).

(iii) Database of adult unrelated (AUD) and cord blood units (CBUs) (optional).

(iv) HLA nomenclature code lists.

(v) Allele and haplotype frequencies (optional, depending on type of the algorithm).

The algorithm itself usually follows the following step.

(a) *Preprocessing*: fast preselection of donors based on predetermined internal indices.

(b) *Processing*: comparison of every (preselected) donor with the patient, calculation of match grades, matching probabilities, and filtering.

(c) *Postprocessing*: linking corresponding donor/CBU details.

The search output, which returns a sorted list of potential donors and CBUs can be presented either in the user interface, on a printed report, or transmitted to other systems (EMDIS). The presentation output may be calculated within the search engine software. For example, it is common practice to highlight patient-donor HLA mismatches as well as match grade and matching probability this may require additional data extraction from internal information calculated during the execution of the algorithm.

2.1. Patient's Data. Patient's HLA typing data must correspond to the valid HLA nomenclature and WMDA guidelines [3] and should be typed at the highest possible resolution, that is, at least intermediate resolution. Some algorithms may return unexpected search results, if low-resolution HLA typing data is provided.

Example 1. B∗35 : 76 has no mapping to "Unambiguous Serology" [4], but is mapped to "Possible Serology" B35 and B22. B22 is the broad HLA code with splits B54, B55, and B56. Therefore, a patient carrying B∗35: XX is a potential match with a donor carrying B56. Such a result is likely to be confusing for healthcare professionals. This problem would not appear if the patient was typed at higher resolution (the B∗35 : 76 allele is excluded). An alternative solution would be to apply an exceptions or filter by application of additional criteria, for example, matching probabilities with threshold (it is very unlikely that B∗35: XX will become B∗35 : 76).

2.2. Patient's Match Criteria. Some algorithms have hard-coded or fixed match criteria, but more sophisticated search algorithms allow users to define matching preferences for each individual search. EMDIS Matching Preferences [5] define the following criteria.

(i) Counting method for mismatches: count graft-versus-host (GvH) mismatches only or host-versus-graft (HvG) mismatches only.

(ii) Maximum number of antigen/allele mismatches for adult donors.

(iii) Maximum number of antigen/allele mismatches for CBUs.

(iv) Maximum number of antigen/allele mismatches at loci A/A∗, B/B∗, Cw/C∗, DR/DRB1∗, DQ/DQB1∗.

(v) Additional sorting criteria like age of the donor, gender matching, and CMV matching.

2.3. Database of Donors and Cord Blood Units (CBUs). Database of unrelated stem cell donors and CBUs should correspond to the following requirements [6].

(i) *Current*: the data used by the algorithm should be up to date.

(ii) *Detailed*: the data presented should contain all relevant fields to the determination of match. The set of data elements should be consistent amongst the registry community.

(iii) *Integrated*: the data presented should be considered as a set and should be available to the matching party as part of a singular search event.

(iv) *Recognizable*: the data presented should uniquely reference individual sources using the identifier that is directly associated with the donor/CBU or would appear on any biological samples associated with the product.

(v) *Comprehensive*: the data presented should represent a consolidated view of the inventory. Uniform depth of access to all donors is needed.

Good implementation of the donor database is essential for acceptable performance of the search algorithm. Not all

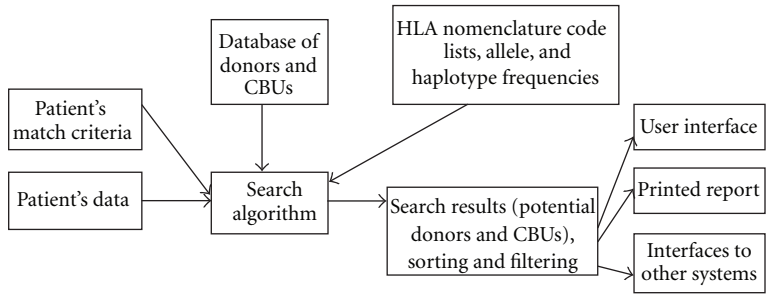

Figure 1: Basic concept of the donor search algorithm.

database structures of HLA applications are suitable as the data source for the algorithm.

Many small to middle size registry are colocated in a single centre with the HLA typing laboratory and there is a need for data integration of these two departments. It may seem that the registry system stores and manages the HLA typing results in the same way as the HLA laboratory information management system (LIMS), and some registries have implemented such data storage. It is a mistake to use these in search algorithms. The main differences between registry database and HLA LIMS database are as follow.

(i) The registry system needs fast access to the most current and comprehensive HLA typing results, which does not always mean the last test typing. This may be combination of multiple tests performed in the past by multiple typing techniques. The registry system always needs access to the full set of all loci that should be stored at one place, while the HLA lab system order includes only requested tests and loci, so HLA typing results of an individual may be spread in multiple typing orders.

(ii) When the HLA lab supervisor approves the order results, it cannot be changed in the lab system. However, the registry system has to keep historical HLA typing results up to date according to the latest HLA nomenclature, so it needs to update them (deleted and renamed alleles, new HLA nomenclature).

Database of donors/CBUs can simply be organized in a single relational database table. Even this may be problematic. A logical database approach is to organize HLA code-lists in separated tables (multiple-allele codes, alleles, antigens, and their relations) and define master-detail relationship between donor data and HLA codes. These systems have been implemented in some registries. The storage of donor record is using only primary keys of HLA codes (as foreign keys). The disadvantage of the master-detail storage is that the retrieval of donor's HLA typing is inefficient. Often the solution for data retrieval in such a structure is cumbersome, because the database system has to join data (database natural join) from tens of tables or do tens of joins of the same table. The advantage is easy manipulation of the properties of HLA codes or even the renaming of HLA allele codes. But such operations are much less common, compared to data retrieval.

2.4. HLA Nomenclature Code Lists. In all cases, the algorithm has to recognize the description of HLA typing codes (e.g., multiple-allele codes) and relations between HLA codes, especially DNA to serology mapping. Some algorithms even use antigen recognition site matching, amino acid sequences, or nucleotide sequences. It is recommended that code lists and code attributes are downloaded from specialist reference websites [4, 7].

Donors have been typed by various different typing techniques and many of them are registered with HLA serological assignments. The database of donors could be preprocessed, so all interpretations and mapping of HLA codes could be saved in advance, but generally, the patient's HLA type is known only at the time of the search, so HLA nomenclature code lists are needed. Of some concern is that a minority of patients are still typed only by serologic typing techniques! This means that search algorithms must be capable of using these in the search process.

3. Preprocessing

Several variants of search algorithms are being used by stem cell donor registries. Selection of the algorithm is influenced by available resources, size of the donor database, availability of haplotype frequencies of the supported population(s), and so forth. We will discuss commonly used search algorithms.

3.1. Simple Preselection. The goal of the algorithm is to find potential donors for one patient. The phenotype of the patient is compared with all donors phenotypes in the donor registry database that are "available" for transplantation purposes (simple preselection).

```
For every donor D in the database
    Count Match Grade (patient P-donor D)
    If the Match Grade is acceptable, store
        data of donor D in the list of
        potential donors of patient P.
```

This kind of algorithm is usually used only for small to middle sized registries. Implementation enhancements can help to improve this situation. For example, increasing current capacities of server memories allows caching of all donors in the random access memory (RAM) of the server. The advantage of this algorithm is mainly in its simplicity

and simple validation process. It also has very straightforward implementation of distributed or parallel computing. The drawback is the speed and memory limitation, especially where donor database is growing.

This algorithm could be extended to multiple patient searches that might be useful, for example, for EMDIS repeat searches [5], when search results from several thousands of donors have to be generated and compared with previous results. Again, the list of all patients could be cached in the server memory with one additional loop.

```
For every donor D in the database
    For every patient P in the database
        Count Match Grade (patient P-donor D)
        If the Match Grade is acceptable, store
            data of donor D in the list of
            potential donors of patient P.
```

3.2. Search Determinants. Databases from Registries and cord blood banks store the HLA types in many formats depending whether typing was by serology or by DNA-based methods. Registries must take these different assignments to create a match algorithm to search for a patient. This comparison is usually facilitated by the conversion of phenotypes to "search determinants" prior to development of matching algorithms [8].

The phenotype of the patient/donor is mapped to "Search Determinants" (SD) [9, 10]. The SD is a data record, based on serological antigens, corresponding to the original HLA phenotype. For example, it might be a group of six HLA, serologic-based assignments—three pairs for HLA-A, HLA-B, and HLA-DRB1 loci. There are also a number of issues with this approach, since some alleles have multiple or no serologic specificities. Therefore, an individual can have multiple SDs. SDs are used as an index to select the set of matching phenotypes. Then, more precise match grades are counted and the list of donors is filtered.

The main application of SDs is the speeding up of the match process by using SDs as key values in conjunction with a database and a matching algorithm [11]. The main disadvantage is the need for regular checks and updates of SDs of all donors in the database; due to changes of donor data, HLA nomenclature updates and changes in the "DNA to serology" mapping. There are particular problems where there is no serological equivalent for a DNA allele.

3.3. DNA Matching Only. The National Marrow Donor Program (NMDP) in the United States has developed an algorithm [12] that does not use SDs for the initial matching step as this is done by directly comparing patient DNA type to donor DNA type. The algorithm is able to account for all serologic typing possibilities with the use of a special table called the "Serology to DNA Allele Table." This table can be generated from the "rel_dna_ser.txt" and "rel_ser_ser.txt" files from hla.alleles.org [4].

4. Processing

The key element of the processing step of the algorithm is the "match grade function" that can compare data (HLA, ethnic group) of two individuals (usually patient and donor) and return their match grade and/or matching probabilities (see Figure 2). The threshold function then filters out donors that do not match patient's match criteria.

Original versions of matching algorithms compared HLA typing only at HLA-A and HLA-B loci. DNA typing was not performed. Later generations added other loci, especially HLA-DRB1, but also HLA-C and HLA-DQB1. Today, some algorithms even use HLA-DRB3/4/5, HLA-DPB1, and other loci.

Earlier versions of matching algorithms also used only serological assignments; DNA typing either did not exist or was not taken into account. Later versions have converted DNA typing results into serological assignments or vice versa, so the algorithm has a uniform typing technique view on all donors. Current search algorithms use DNA typing results as much as possible and switch to serology comparisons only if DNA typing is not provided or if they want to refine DNA to serology mapping.

The Information Technology (IT) Working Group of the World Marrow Donor Association (WMDA) has issued two key resources that describe the correct handling of HLA data and key patient-donor matching procedures:

(i) framework for the implementation of HLA matching programs in hematopoietic stem cell donor registries and cord blood banks [2]. This paper gives a bottom-up approach to the design of search algorithms: comparison of individual HLA codes, then HLA single-locus phenotypes, and eventually HLA multilocus phenotypes;

(ii) fuidelines for use of HLA nomenclature and its validation in the data exchange among hematopoietic stem cell donor registries and cord blood banks [3].

A common mistake in the design of search algorithm is the violation of the rule 2.1 of the guidelines [3]: "laboratories must assign DNA nomenclature to results obtained using DNA-based methods and serologic nomenclature to results obtained using antibody reagents." Some computer systems need to permanently store serology-derived results of DNA codes, usually because of simple DNA-serology matching. However, the mapping should be done automatically by the system and not by the user. Derived serology values must be clearly distinguished from real serology results obtained using antibody reagents. Where mapping has changed, the registry system has to know if stored serologic results should be updated or not. Moreover, some alleles are mapped to multiple serology equivalents and the system has to take this into account.

In addition to match grade, some information can be calculated. In these, the probability of HLA matching at the allele level based on local population haplotype frequencies in the underlying population can be calculated. Such prediction algorithm system has been developed and validated by the NMDP (HapLogic II) [13].

The latest, state-of-the-art versions of search algorithms (OptiMatch, HapLogic III) use these probability calculations to determine the rank order of HLA matches as the main searching and sorting criteria.

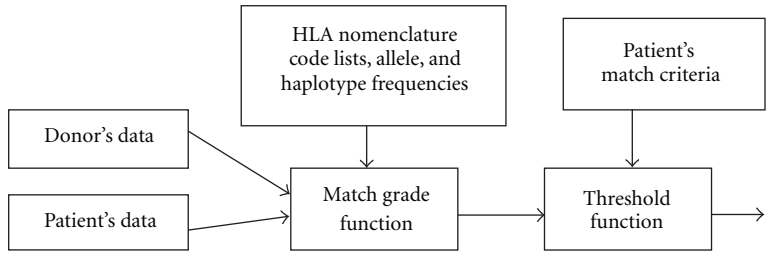

Figure 2: Match grade function.

5. Postprocessing

At this stage, the system retrieves corresponding donor details of all selected donors that will be displayed in the search results. If the matching probabilities are not used as the main sorting criteria, the search system can apply them at this stage (ProMatch [14], Hap-E [15] and EasyMatch [16]).

6. Probability Matching Algorithms

The search algorithms of the two largest registries in the world are based on the probability matching approach.

Using a large number of high resolution HLA types the system can estimate the probability of other less well-typed donors being matched to the patient. The system is validated by retyping these donors to obtain high resolution types/haplotypes and thereby confirming that the calculation is accurate. The limitation of this is that it may be specific to an ethnic group.

6.1. OptiMatch. OptiMatch [17] is a matching program calculating, for each donor, the probability of allelic identity to the patient. The program was developed by the German registry ZKRD. The first version (October 2006) was based on 3 locus high resolution haplotype frequencies, while the current version (June 2008) is based on 5 locus high resolution haplotype frequencies.

The web-based user interface lists potential donors with 7 probabilities: A∗ match, B∗ match, C∗ match, DRB1∗ match, DQB1∗ match, and overall probabilities of 10/10 match and 9/10 match.

6.2. HapLogic. The HapLogic program [13, 18] was developed by the NMDP registry. It works in a similar way to OptiMatch. First versions calculated probabilities of 6/6 allele matches, while the latest version III, introduced in November 2011, sorts donors based on probability of matching 10 alleles, using 5 locus high resolution haplotypes (like OptiMatch). HapLogic also uses 5 broad and 21 detailed race/ethnic groups.

The web-based user interface shows a list of potential donors with several probabilities: A∗ match, B∗ match, C∗ match, DRB1∗ match, DQB1∗ match, and overall probabilities of 10/10 match, 9/10 match, 8/10 match, 8/8 match, 7/8 match, 6/8 match, and for cord blood units also 6/6 match, 5/6 match, and 4/6 match.

7. Implementation of the Probability Matching Algorithm

If the registry wants to implement probability matching algorithm, such as OptiMatch or HapLogic, it has to successfully complete the following three steps.

(1) Design and implement the algorithm itself.

(2) Estimate haplotype frequencies of the donor (and patient) populations—these 5 locus high resolution haplotype frequencies are usually estimated from a donor registry database.

(3) Validate the search system—using retrospective data of historical searches. Usually, registry confirmatory typing requests (CTs) and their results are used.

There are two potential problems with the development of this approach: (1) unlike ZKRD and NMDP, other registries do not have sufficient donors to estimate 5 locus high resolution haplotype frequencies. Haplotype frequencies could be calculated, but their confidence is questionable. (2) Smaller registries also do not have enough high resolution HLA types (obtained at confirmatory typing, CT) for validation of the prediction algorithm. ZKRD used 9843 CTs in 2008 [17] and 22255 CTs in 2010 [19]. NMDP used about 60 000 CTs (not published). These numbers are not achievable in smaller registries.

In order to overcome these problems, the Prometheus system approximated the local population to the German (ZKRD) population, that is, by using ZKRD high resolution A∗-B∗-C∗-DRB1∗-DQB1∗ haplotype frequencies [14]. It also used high resolution HLA types from CT samples from multiple registries.

8. Validation of the Search Algorithm

All implementations of the search algorithms need to be validated before being used. The WMDA Information Technology Working Group provides validation sets of patients and donors that are used for matching trials and comparison of results with expected outcomes [2, 20]. Algorithms that do not use simple preselection approach, but use more complex preselection, have to be validated for completeness. It is important not to miss any relevant donors in the preselection [2].

Validation of the processing phase, especially the match grade function, can be done by running several automated

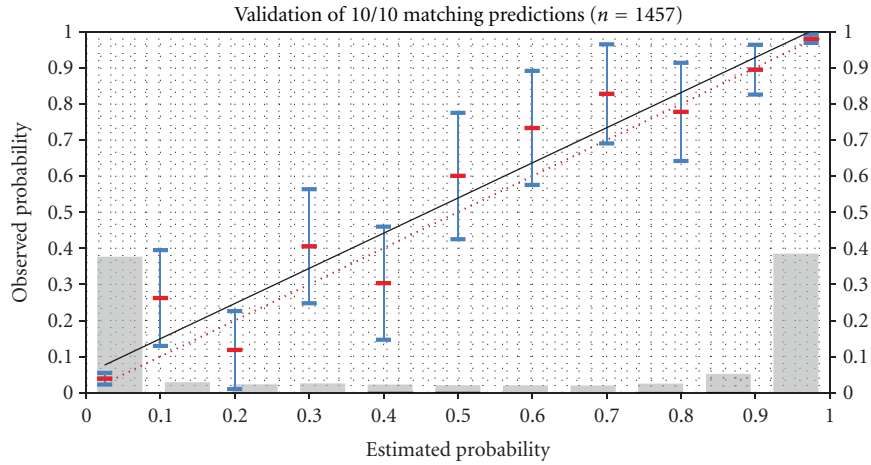

Figure 3: Prometheus probability matching algorithm (ProMatch): the graph shows the correlation of estimated 10/10 matching probabilities in 10% prediction intervals and corresponding observed probabilities. The population model is approximated by the German population. Blue bars show 95% confidence intervals of estimated probabilities. Grey bars show relative number of CTs in each prediction interval. Red-dotted line is the ideal correlation.

unit tests, addressing all kinds of matches and mismatches, exceptions, and rare cases. Interfaces to software source code classes, modules, or libraries are tested with a variety of input arguments to validate that the results that are returned are as expected [21].

The quality of prognostic matching algorithm and the population model used (allele and haplotype frequencies) also has to be validated. This is usually done by retrospective or prospective studies. Typically, all CTs performed by the registry that meet some criteria are used. These criteria are as the follows.

(i) Patient has been typed in high resolution.

(ii) Donor was not typed in high resolution before the CT, but has been high resolution typed at the time of CT (or later).

(iii) No discrepancy between a priori and final HLA type.

The review process retrospectively calculates the matching prognosis and compares the predicted and observed percentage of allele matches (see Figure 3).

9. Conclusions

A reliable and efficient search algorithm is the key component of the unrelated stem cell donor registry computer system. An overview of search algorithms, their design, and implementation aspects have been described. Both combinatorial and probability matching algorithms have been presented.

A top-down design approach that first lists algorithm requirements, specifies input and output parameters, and then goes deeper into details was selected. The importance of validation prior to the implementation of a new matching algorithm has been emphasized.

Conflict of Interests

The author declares no conflict of interests.

Acknowledgments

Karel Peyerl and Ann Green have provided valuable input to this paper. Yves Garcia from One Match and Matt Prestegaard from NMDP are authors of the document entitled "Unrelated Hematopoietic Stem Cell Donor Search and Facilitation Information Systems Principles" ("2012-03-15 World Match Central Glossary of Terms.docx"). The author has used their definition of terms "Consistently matched," "Current," "Detailed," "Integrated," "Recognisable," and "Comprehensive." Research described in the paper has been supported by the research Program no. MSM 6840770012 "Transdisciplinary Research in Biomedical Engineering II" of the CTU in Prague.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 539825, 7 pages
doi:10.1155/2012/539825

Clinical Study

The Presence of Anti-HLA Antibodies before and after Allogeneic Hematopoietic Stem Cells Transplantation from HLA-Mismatched Unrelated Donors

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Received 26 June 2012; Revised 17 September 2012; Accepted 18 September 2012

Academic Editor: Bronwen Shaw

Although anti-human leukocyte antigen antibodies (anti-HLA Abs) are important factors responsible for graft rejection in solid organ transplantation and play a role in post-transfusion complications, their role in allogeneic hematopoietic stem cell transplantation (allo-HSCT) has not been finally defined. Enormous polymorphism of HLA-genes, their immunogenicity and heterogeneity of antibodies, as well as the growing number of allo-HSCTs from partially HLA-mismatched donors, increase the probability that anti-HLA antibodies could be important factors responsible for the treatment outcomes. We have examined the incidence of anti-HLA antibodies in a group of 30 allo-HSCT recipients from HLA-mismatched unrelated donors. Anti-HLA Abs were identified in sera collected before and after allo-HSCT. We have used automated DynaChip assay utilizing microchips bearing purified class I and II HLA antigens for detection of anti-HLA Abs. We have detected anit-HLA antibodies against HLA-A, B, C, DR, DQ and DP, but no donor or recipient-specific anti-HLA Abs were detected in the studied group. The preliminary results indicate that anti-HLA antibodies are present before and after allo-HSCT in HLA-mismatched recipients.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment of both congenital and acquired disturbances of hematopoiesis, especially of hematological malignancies.

The selection of the optimal donor is based on high-resolution HLA typing. The MHC (Major Histocompatibility Complex) contains more than 200 genes which are situated on the short arm of chromosome 6 at 6p21.3. It is divided into three main regions: HLA class I (containing *HLA-A*, *B*, and *C* genes), class II (containing *HLA-DR*, *DQ*, and *DP* genes), and class III region. The role of HLA molecules is to present peptides to T cells (both CD4 and CD8 T cells), enabling them to recognize and eliminate "foreign" particles and also to prevent the recognition of "self" as foreign. HLA mismatches may occur at antigenic or allelic level; the first are characterized by amino acid substitutions in both peptide-binding and T-cell recognition regions, whereas the latter are characterized by amino-acid substitution in the peptide binding regions only [1].

HLA antigens are recognized by immunocompetent T cells, what may lead to graft failure, graft versus host disease (GVHD), and other posttransplant complications as well as to favorable graft versus leukemia (GVL) effect. HLA molecules bear multiple antigenic epitopes, many of which are the so-called "public" epitopes that are shared among the products of several different HLA alleles, resulting in the apparent cross-reactive groups of antigens (CREGs). These shared epitopes may be responsible for patient's sensitization

to multiple HLA antigens, despite a single antigen mismatch only [2–4].

The participation of cellular arm of immunological response to HLA antigens is well known, but the role of humoral arm of immunity is also very interesting, especially when we consider the enormous polymorphism of HLA-genes, their immunogenicity and huge heterogeneity of antibodies. Antibodies are glycoproteins that belong to the superfamily of immunoglobulins [5]. The basic structural units of antibodies are two heavy chains (α, γ, ε, δ or μ) and two light chains (κ or λ). The type of a heavy chain determines the class of antibody: IgA, IgG, IgM, IgE or IgM [6]. The region of chromosome that encodes the antibody is large and contains several distinct genes. The locus containing heavy chain genes is found on chromosome 14; loci containing κ and λ light chain genes are found on chromosomes 2 and 22, respectively. The enormous diversity of antibodies allows the immune system to recognize an equally wide variety of antigens [5]. It has been known that humans produce about 10 billion different antibodies capable of binding a distinct epitope of an antigen [7]. Such a diversity of antibodies is caused by domain variability, recombination, somatic hypermutation and affinity maturation, class switching, and affinity designations [8–10]. Anti-HLA Abs may be present in healthy individuals [11, 12]. The sensitization to MHC antigens may be caused by transfusions, pregnancy, or failed previous grafts [13]. Anti-HLA Abs are more frequently detected in patients with hematological disorders due to their alloimmunization, resulting mainly from common use of transfusions [14].

The clinical significance of anti-HLA Abs is well known in the field of transfusional medicine. The presence of anti-HLA Abs in patients is one of the major causes of platelet transfusion refractoriness [15]. On the other hand, anti-HLA Abs present in blood products have been shown to be a major cause of transfusion-related acute lung injury (TRALI) [16, 17]. The role of anti-HLA Abs is also well known in solid organ transplantation—especially in kidney transplantation, because transplanted kidneys are highly susceptible to antibody-mediated injury [18, 19]. Antibodies produced before kidney transplantation (reacting with donor's HLA antigens) induce hyperacute or acute vascular rejections which frequently result in transplant failure [20, 21].

Despite the well-recognized role of antibody-mediated rejection in solid organ transplantation, the graft rejection following allo-HSCT is generally attributed to cytolitic host-versus-graft (HVG) reaction mediated by host T and NK cells, that survived the conditioning regimen [22–25]. Engraftment failure rate is approximately 4% in allo-HSCT from matched unrelated donor (MUD) and about 20% in cord blood or T-cell-depleted haploidentical transplantations [26, 27]. Antibody-mediated bone marrow failure after allogeneic bone marrow transplantation can be also caused by antibody-dependent cell-mediated cytotoxicity (ADCC), or complement-mediated cytotoxicity [28–30]. In ADCC, the cytotoxic destruction of antibody-coated target cells by host cells is triggered when an antibody bound to the surface of a cell interacts with Fc receptors on NK cells or macrophages. Preformed antibodies present at the time of hematopoietic stem cell infusion are unaffected by standard transplantation conditioning regimens, T- or B-cell immunosuppressive drugs or modulatory strategies given in the pretransplantation period [31].

Albeit the T-cell-mediated cellular immunity is the primary barrier for bone marrow allorejection in nonsensitized recipients in the animal models (mice), the humoral arm of the immune response plays a very important, previously unappreciated, role in the rejection of allogeneic stem cell transplantation in sensitized mice and in such case the rejection of a bone marrow is T-cell independent [31, 32]. Moreover, the achievement of a mixed allogeneic chimerism resulted in reverse of the sensitization in allosensitized recipients [30, 33]. Probably not only antigen-specific but also cross-reactive or broadly reactive alloantibodies may be responsible for the graft failure [32]. Spellman and Bray have demonstrated in a retrospective, case-controlled study that the prevalence of donor-specific anti-HLA antibodies was higher in a group of mismatched unrelated donor recipients who suffered graft rejection than in a control group that engrafted. Among the 37 recipients who failed to engraft 9 (24%) possessed DSAS against HLA-A, B, or DP, but only 1 (1%) recipient of 78 controls did [34]. In the study of Ciurea et al. DSAS was the single most important factor associated with graft failure and HLA-mismatches increased the occurrence of donor-specific HLA antibodies in MUD transplantation [35]. Takanashi et al. demonstrated the impact of anti-HLA antibodies on engraftment after myeloablative single unit cord blood transplantation. Patients with anti-HLA antibodies experienced slower neutrophils and platelet recovery than antibody-free patients. Although no effect of anti-HLA antibodies on GvHD grade II-IV, relapse, or TRM has been observed, the overall and event-free survival were significantly inferior in antibody-positive patients [36]. Similar observations were made after double umbilical cord blood transplantation [37].

As presented above, the influence of anti-HLA Abs, including Abs directed against mismatched antigens, on the results of allo-HSCT, especially on graft failure, has been proved in several reports. However, in patients following allo-HSCT, the series of time remote complications may occur. As antibodies appearing in the result of the earlier immunization are detected before transplantation, the question of their presence and specificity after transplant, after the myeloablative conditioning treatment, and during administration of immunosuppressive therapy is open, when the hematopoietic function is taken over by the donor's cells. The first cells to reconstitute (within the first 100 days) after the transplantation are granulocytes, monocytes, macrophages, and NK cells. In contrast, T and B lymphocytes remain severely reduced and their function is impaired from 6 months to 1 year after the transplantation [1].

Therefore, the aim of our study was to examine the presence and the specificity of anti-HLA antibodies before and following the allo-HSCT.

2. Materials and Methods

We included 30 patients who received allo-HSCT from partially HLA-mismatched unrelated donors and who agreed

to participate in the study. Donors lacking full HLA compatibility with recipients were chosen when compatible donors were not available. Standard high-resolution allelic typing of HLA-A, B, C, DRB1, and DQB1, without HLA-DP, was performed. The study was carried out in the Department of Hematology and Bone Marrow Transplantation of the Medical University of Silesia in Katowice, Poland, between 2007 and 2011. The examination of patient's sera was scheduled at 4 time points: before the start of conditioning treatment and 30 days, 100 days, and 1 year after transplant.

The preparative treatment was myeloablative in 28 (93%) and reduced in 2 (7%) pts. Standard GVHD prophylaxis consisted of pretransplant antithymocyte globulin, cyclosporine A in initial dose 3 mg/kg i.v. starting from day −1 with dose adjusted to its serum level and shift to oral administration about day +20, methotrexate 15 mg/m^2 i.v. on day +1, and 10 mg/m^2 i.v. on days +3 and +6. Methylprednisolone at dose 2 mg/kg i.v. was the first line therapy of aGVHD symptoms; in few patients mycophenolate mofetil or tacrolimus was used. The source of cells was the bone marrow in 9 (30%) and peripheral blood in 21 (70%) patients.

The detailed characteristics of the study population are presented in Table 1.

Patient's sera were tested for the presence of anti-HLA Abs in the HLA and Immunogenetics Laboratory of Regional Blood Center in Katowice, Poland. Anti-HLA A, B, C, DR, DQ, and DP antibodies were detected and identified using the ELISA-based DynaChip Technology. The DynaChip HLA Antibody analysis system utilizes microchips spotted with purified HLA antigens immobilized on the surface of the glass chip. Test serum was free of aggregates and excess lipids before testing. This was achieved by centrifugation for 10 minutes at 10,000 g. The clarified supernatant was diluted with the Sample Diluent contained within the kit and then it was added to the DynaChip wells. Anti-HLA Abs present in the test serum were bound to the HLA antigens on the surface on the chip. Bound antibodies were then detected using the Antibody Detection Reagent (antihuman IgG and horseradish peroxidase complex). The assay was completed with colorimetric detection. The resulting patterns of blue-positive and clear-negative spots were recorded by the software and subsequently automatically analyzed by the DynaChip Analysis Software. The presence of at least one anti-HLA antibody was regarded as presence of anti-HLA Abs, whereas if the examined serum contained antibodies against more than 50 different HLA antigens they were regarded as "anti-HLA Abs to many specificities." Applied DynaChip HLA Antibody analysis system did not allow to measure the concentration of detected antibodies.

The study has been approved by the responsible Ethical Committee of Medical University of Silesia.

3. Results

Anti-HLA Abs were detected in 26 (86.6%) patients. Anti-HLA Abs against HLA class I, II, or both were detected in 8 (26.6%), 2 (6,6%), or 16 (53.3%) patients, respectively. In 4 (13.3%) patients they were detected before transplant only,

Table 1: Patients characteristics ($n = 30$).

Median age (range)	
Recipient	37 (13–57) years
Donor	36 (19–55) years
Mean time from diagnosis to allo-HSCT (range)	0.75 (0.63–10.3) years
	Number (%)
Sex	
Donor	
Male	19 (63.3%)
Female	11 (36.7%)
Recipient	
Male	16 (53.3%)
Female	14 (46.7%)
Sex matching	
Male donor, male recipient	10 (33.3%)
Female donor, female recipient	5 (16.6%)
Male donor female recipient	9 (30%)
Female donor, male recipient	6 (20%)
HLA- mismatch	
Antigen A	4 (13.3%)
Antigen C	12 (40%)
Antigen DQ	2 (6.6%)
Allele A	2 (6.6%)
Allele B	5 (16.6%)
Allele DQ	3 (10%)
Antigen B + Antigen C	1 (3.3%)
Antigen A + Allele B	1 (3.3%)
Primary indication for allo-HSCT	
Acute lymphoblastic leukemia (ALL)	6 (20%)
Acute myeloid leukemia (AML)	15 (20%)
Chronic myeloid leukemia (CML)	5 (16.6%)
Chronic lymphocytic leukemia (CLL)	1 (3.3)
Severe aplastic anemia (SAA)	2 (6.6%)
Paroxysmal nocturnal hemoglobinuria (PNH)	1 (3.35)
Preparative regimen	
Cyclophosphamide	1 (3.3%)
TBI + Cyclophosphamide	6 (20%)
TBI + Fludarabine	1 (3.3%)
Treosulfan + Fludarabine	6 (20%)
Busulfan + Cyclophosphamide	12 (40%)
Busulfan + Fludarabine	1 (3.3%)
Treosulfan + Cyclophosphamide	1 (3.3%)
Busulfan + Cyclophosphamide + Gemtuzumab Ozogamycin	1 (3.3%)
Rituximab + Alemtuzumab + Melphalan	1 (3.3%)
Immunosuppressive treatment	
Glycocorticoid	27 (90%)
Cyclosporine	30 (100%)
Mycophenolate mofetil	7 (23%)
Tacrolimus	1 (3.3%)
Source of cells	
Bone marrow	9 (30%)
Peripheral blood	21 (70%)

Table 2: Anti-HLA antibodies detected before and 30 days, 100 days and 1 year after allo-HSCT in 30 recipients.

No	Typing of mismatched HLA		HLA-mismatch	Detected anti-HLA Abs with regard to allo-HSCT			
	Recipient	Donor		Before	+30 days	+100 days	+1 year
1	C 0401 C 0501	C 12XX C 0501	Antigen C	Many specificities	Not tested	DR15	Not tested
2	DQB1 0202 DQB1 0302	DQB1 0202 DQB1 0301	Allele DQB1	Many specificities	Not tested	B70	Not tested
3	C 0802 C 1203	C 0802 C 0303	Antigen C	Many specificities	Not tested	Not tested	Not detected
4	C 0501 C 0702	C 0201 C 0702	Antigen C	Not detected	B65, B46, B37, C36, C10	DRB1*15, DQB1*06	Not detected
5	A 0201 A 0302	A 0201 A 0301	Allele A	Not detected	Not detected	Not tested	C14, B62, C9, A26
6	C 0401 C 1602	C 0401 C 1502	Antigen C	Not detected	Not tested	Many specificities	Not detected
7	C 0802 C 1502	C 0702 C 0702	Antigen C	Not detected	Not tested	Many specificities	Not detected
8	DQB1 0202 DQB1 0301	DQB1 0303 DQB1 0301	Antigen DQB1	Not detected	Not tested	Not detected	Not detected
9	C 0303 C 0102	C 0403 C 0102	Antigen C	Not detected	Not tested	DR13	B82, B49
10	C 0303 C 0401	C 0401 —	Antigen C	A23, A24, B27, B35, B38, B40, C0803, C0804	Not tested	B45, A66	DQ8, DR4
11	C 0303 C 0602	C 0403 C 0602	Antigen C	B75, B46, DR13, DQ3	Not tested	Not detected	Not detected
12	DQB1 0301 DQB1 0504	DQB1 0301 DQB1 0501	Allele DQB1	Not detected	Not tested	Not detected	B82, A34, DQ8, DR4
13	C 0202 C 0102	C 0202 C 0202	Antigen C	Not detected	Not tested	Not tested	B70
14	DQB1 0602 DQB1 0602	DQB1 0602 DQB1 0603	Allele DQB1	Many specificities	Many specificities	Not detected	Not detected
15	C 0102 C 0602	C 0302 C 0602	Antigen C	Not detected	C18, DRB3*, DPB1*05, DRB104, DQB1*06	Not tested	Not tested
16	A 1101 A 2601	A 24xx A 2601	Antigen A	Not detected	Not detected	Not tested	DQ2, DQ4, DQA02, DQA04
17	A 0205 A 2402	A 0201 A 2402	Allele A	DQB1*03, DRB1*04	Not detected	Not tested	Not tested
18	B 4102 B 5601 C 0401 C 1703	B 4102 B 5501 C 0301 C 1703	Antigen B, antigen C	Not detected	Not detected	Not detected	Not tested
19	A 2601 A 3201	A 0201 A 3201	Antigen A	DQ8, DR4	DQ8, DR4	Not detected	B45, A66, DR10, DR12
20	DQB1 0301 DQB1 0302	DQB1 0301 DQB1 0402	Antigen DQB1	A2, 2C A0302, B67	A2, C2, B67	A2, DR 16	Not tested
21	A 0201 A 2901	A 0205 A 2901	Allele A	B7, 7C, B60, B81, A2403, A2608, C0727, C0804	B7	B47, B63	Not tested

Table 2: Continued.

No	Typing of mismatched HLA		HLA-mismatch	Detected anti-HLA Abs with regard to allo-HSCT			
	Recipient	Donor		Before	+30 days	+100 days	+1 year
22	C 0501 C 1203	C 0501 —	Antigen C	Not detected	Not detected	Not detected	Not detected
23	C 0304 C 0702	— C 0702	Antigen C	A2 A0302, B2703, B3501, B3503, 4006, B4101, B45, B4604, B67, B76, B78, C0103, C0403, DR51, DR15, DQ6, DR16, DQA01 DPB39, DPB3901, DPB85, DPB8501, DQB0502	A2, A0207, A0302, B2703, B3503, B4006, B4101, B67, B76, C0403, DR51, DR15, DQ6, DR16 DPB39, DPB3901, DPB85, DPB8501, DQB0502	A2, DR51, DR16	DQ7, DQA05
24	B 3501 B 5701	B 3503 B 5701	Allele B	B42, A80, C17	Not detected	B77	Not detected
25	A 2402 A 2601	A 03xx 2601	Antigen A	Not detected	Not detected	Not detected	Not tested
26	B 3501 B 3502	B 3501 B 35xx	Allele B	C7, DQ8	Not detected	Not detected	Not detected
27	A 3001 A 3101	A 01xx —	Antigen A	DR10, DR11	B77, B38	Not detected	Not tested
28	A 2501 A 3201	A 2501 A 23xx	Antigen A	Not detected	B77, A36	Not detected	Not detected
29	B 1801 B 4402	B 1801 B 4427	Allele B	A31	Not detected	B61, C15, B35	Not tested
30	B 3503 B 3501	B 3503 B 3503	Allele B	DQ5, DQ6, DQA01	C7, DQ6, DR51, DPB39, DPB3901, DPB85, DPB8501, DQB0502,	C7, DR 51, DPB14, DPB1401, DQB0502, DQB0602, DQB0608, DR0806, DR2, DQ6, DQA01	Not tested

in 10 (33.3%) patients after transplant only, and in 12 (40%) patients both before and after transplantation. In 4 (13.3%) patients anti-HLA Abs were not detected neither before nor after allo-HSCT. Anti-HLA Abs directed against the class or antigens of mismatched HLA were detected in 4 patients before transplant and in 9 patients after transplant. In 5 patients we identified antibodies with the same specificities before and 30 days after the transplantation (as presented in Table 2, cases' numbers: 19, 20, 21, 23, and 30). Although we did not identify neither donor or recipient allele-specific anti-HLA Abs, antibodies that detected after transplant in 3 patients belonged to the same CREG (Cross-Reactive Group) as recipient's mismatched HLA antigen (as presented in Table 2, cases' numbers: 19-10CREG, 24-5CREG, and 29-12CREG). These antibodies were detected more than 100 days after transplantation, so it is very likely that they were produced by donor cells.

The specificities of anti-HLA Abs detected before allo-HSCT and at different time-points after transplant are presented in Table 2. We have succeed only partially in consequent collecting sera at all scheduled timepoints from patients included into the study for analysis due to the fact that some patients were referred to our center for allo-HSCT from remote parts of Poland. After allo-HSCT they have moved for care to their home centers and collection of the complete set of sera from them was impossible.

4. Conclusions

Our preliminary results indicate that preformed anti-HLA Abs can be detected before and may also appear after transplant in mismatched allo-HSCT recipients. Anti-HLA Abs present in 3 patients were directed against HLA antigens which belonged to the same serological Cross Reactive Groups as the mismatched HLA antigens.

In 5 patients anti-HLA Abs directed against the same HLA antigen were detected before and after allo-HSCT what may indicate that they were not destroyed during the myeloablative conditioning treatment and standard immunosuppressive therapy. These antibodies belonged to the same serological Cross Reactive Group as the recipient's but not donor's mismatched HLA antigens, so it is possible to conclude that donor's cells may produce anti-HLA Abs against the recipients cells after allo-HSCT. Therefore, they may theoretically be responsible for induction of several immunological posttransplant complications. Antibodies detected after transplantation may also result from immunization, for example, by transfusions, as allo-HSCT recipients often require intensive supportive treatment with blood derivatives.

We believe that our observations help to better understand the immune mechanisms contributing to allogeneic sensitization which may influence allo-HSCT results. It is possible that sensitized patients who possess anti-HLA antibodies before or after the transplantation could benefit from modification of conditioning and immunosuppressive therapeutic approaches in the future.

Presented preliminary outcomes of 30 patients are based only on part of our whole study group which consists of 70 patients. The statistical analysis aimed to reveal the eventual impact of anti-HLA Abs on allo-HSCT results will be performed after completion and examination of sera taken at all scheduled timepoints from the whole group. We also consider the extension of the search for anti-HLA Abs with utilization of Luminex Labscreen method which enables to calculate the mean fluorescence intensity and thus to assess the concentration of detected antibodies.

Conflict of Interests

The authors report having no conflict of interests.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 873695, 5 pages
doi:10.1155/2012/873695

Clinical Study

Both Optimal Matching and Procedure Duration Influence Survival of Patients after Unrelated Donor Hematopoietic Stem Cell Transplantation

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Received 28 June 2012; Accepted 16 September 2012

Academic Editor: Colette Raffoux

Eighty-six patients suffering from hematological malignancies, immunodeficiencies, and aplastic anemias received alloHSCT from unrelated donors. Donors were selected from the BMDW files and further matching was performed according to the confirmatory typing procedure with the use of PCR SSP and that based on sequencing. The time from the clinical request of the donor search to the final decision of clinicians accepting the donor was from 0.3 to 17.8 months (median 1.6). Matching was analyzed at the allele level, and 50, 27, and 9 donor-recipient pairs were 10/10 matched, mismatched in one or more alleles, respectively. In an univariate analysis we found better survival if patients were transplanted: (i) from donors matched 10/10 ($P = 0.025$), (ii) not from female donor to male recipient ($P = 0.037$), (iii) in female donation from those with ≤ 1 pregnancy than multiparous ($P = 0.075$). Notably, it became apparent that duration of the confirmatory typing process affected the survival (HR = 1.138, $P = 0.013$). In multivariate analysis only the level of matching and the duration of the matching procedure significantly affected the survival. In conclusion, the duration of the matching procedure in addition to the level of matching should be considered as an independent risk factor of survival.

1. Introduction

The number of allogeneic hematopoietic stem cell transplantations (alloHSCTs) from unrelated donors has increased over the years and in Europe reached 7098 in 2010 (EBMT Survey on Transplant Activity 2010). This was possible due to the improvement in international cooperation in donor-recipient matching procedures facilitated by the Bone Marrow Donors Worldwide (BMDW) files [1] and implementation of the European Marrow Donor Information System (EMDIS) in a number of countries. The priority of the search procedure is to identify the optimally matched donor for patients badly needing hematopoietic stem cell transplantation (HSCT). Quite recently the pace of the matching procedure has improved due to the use of computer-assisted communication systems including the EMDIS. However, still some time is needed, especially when the process of searching for a fully matched donor is prolonged. Previously published studies showed that the time needed to identify an acceptable donor is associated with a profile of HLA alleles being prolonged in cases with rare haplotypes [2–4]. Prolonged search may result in postponing transplantation in some cases that become medically unfit in the meantime. This may be due to various medical reasons including relapse and consequently, unless successfully treated, advancing in the stage of the disease. Tiercy et al. [4] showed that patients categorized in the group with a high probability of finding an optimal 10/10 matched donor have better survival than

Table 1: Patients' characteristics.

Recipient age (y), median (range)		28.5 (0.6–59)
Diagnosis, no. (%)	Hematological malignancy	73 (80)
	Immunodeficiency	9 (15)
	Aplastic anemia	4 (5)
Donor age (y), median (range)		34 (19–59)
Donor-recipient sex match, no. (%)	Female to male	23 (27)
	Other	63 (73)
Number of pregnancies in female donors, no. (%)	0-1	21 (54)
	>1	18 (46)
Donor-recipient CMV serostatus match, no. (%)	Positive-negative	10 (38)
	Negative-positive	26 (72)
Donor origin, no. (%)	Poland registry	14 (17)
	Europe foreign registry	63 (73)
	Other world registries	9 (10)
Donor-recipient HLA matching, no. (%)	Matched	50 (58)
	Mismatched	36 (42)
Number of CT procedures, no. (%)	≤2	58 (73)
	>2	22 (27)
Duration of the matching procedure (mth), median (range)		1.6 (0.27–17.8)
Hematopoietic stem cell source, no. (%)	Bone marrow	6 (7)
	Peripheral blood	80 (93)

those with intermediate or low probability. Here, we study the impact of the actual length of the search procedure on the outcome of alloHSCT.

2. Materials and Methods

2.1. Patients. In this study we analyze the outcome of 86 patients transplanted in our institution from unrelated donors in years 2004–2010. The patients suffered from hematological malignancies (80%), immunodeficiencies (15%), and aplastic anemias (5%). The group consisted of 39 (45%) females and 47 (55%) males aged from 0.6 to 59 years (median 28.5) and received marrow (6) or PBPC (80) from female (40) and male (46) donors (Table 1).

2.2. Histocompatibility Testing and Search Strategy. The donor-recipient matching procedure commissioned to the National Polish Bone Marrow Donor Registry (NPBMDR), a part of the Lower Silesian Center for Cellular Transplantation, was conducted according to two principles: (i) a donor should be compatible in human leukocyte antigen (HLA) with a patient at a high-resolution level of typing considering five loci (A, B, C, DR, and DQ) and (ii) among donors with similar HLA characteristics, residents of Poland, and if absent those from neighboring countries are chosen with priority [5, 6]. Donors were selected from the BMDW files with an HLA-compatible potential with a priority according to the distance principle policy. Further matching procedures were performed as follow: (1) registries having potential donors are conducted to confirm the donor availability and if so a blood sample is requested for confirmatory typing (CT), (2) as soon as blood is received high resolution typing of a potential donor is performed with the use of PCR SSP and that based on sequencing, and the same procedure is applied to the recipient, (3) the transplant center is asked for acceptance of a donor which may result in a request for further search, (4) the above procedures are performed in an iterative manner.

The time from the beginning of the search process, the level of matching and the outcome of transplantation were recorded and statistically evaluated.

2.3. Data Collection. The outcome of transplantation was followed and registered in a database according to the EBMT Med-A form requirements. The overall survival of patients receiving alloHSCT from unrelated donor was evaluated using the already known factors including level of HLA matching, female-to-male donation, number of female donor pregnancies, age of donors and CMV serostatus and in addition the duration of the matching procedure.

2.4. Statistical Analysis. Statistical analysis was conducted using STATISTICA v.10. The associations between two variables were tested by Chi-square test, with Yates' correction if appropriate, for categorical variables and Mann-Whitney U test for categorical and continuous variables. The overall survival was analyzed by the Kaplan-Meier method, log-rank

Table 2: Univariate analysis (discrete variables).

		No.	Overall survival (2-yr survival, %)	*P*-value
Donor-recipient HLA matching	Matched	50	58.9	0.025
	Mismatched	36	37.7	
Donor-recipient sex match	F-M	23	31.8	0.037
	Other	63	56.6	
Number of pregnancies in female donors	0-1	21	52.5	0.075
	>1	18	38.9	
Donor-recipient CMV serostatus match	Positive-negative	10	58.3	0.479
	Negative-positive	26	48.8	

test, and parametric survival models [7, 8]. The likelihood of committing a type 1 error was set to 0.05.

3. Results

All patients were typed at the level of a primary workup in a majority of cases. However, in 15% of cases patients were typed when it was clinically apparent that the transplant was badly needed. The time of the donor search varied from 0.3 to 17.8 months (median 1.6). Analysis of the level of matching at the point of clinical acceptance revealed that 50, 27, and 9 donor-recipient pairs were 10/10 matched, mismatched in one or more alleles, respectively.

The overall survival was significantly higher for patients transplanted from donors matched at the level of 10 specificities (2-year survival rates of matched and mismatched donors: 59% versus 38%, respectively; log-rank test $P = 0.025$) and transplanted other than from female donor to male recipient (2-year survival rates: 57% versus 32%, respectively; log-rank test $P = 0.037$). Survival curves of patients transplanted from female donors with no or 1 pregnancy tended to be higher than those reflecting the effect of donation from multiparous women (2-year survival rates: 53% versus 39%; log-rank test $P = 0.075$).

Notably, it became apparent that duration of the searching process (mth) affected the survival (Cox model: hazard ratio HR = 1.138, $P = 0.013$). The results of univariate statistical analysis are shown in Tables 2 and 3.

In multivariate analysis only the level of matching and the duration of the matching procedure significantly affected the survival in an independent fashion (Cox model: HR = 2.422, $P = 0.007$ and HR = 1.109, $P = 0.045$, resp.) (Table 4). Multivariate analysis was used to calculate the coefficients reflecting the impact of different variables on the overall survival. More thorough analysis of the study group revealed that the duration of the searching process was significantly longer in patients having as compared to those lacking the presence of rare haplotypes and/or rare B-C or DR-DQ associations defined according to our published study (median: 3.1 versus 1.5 months, Mann-Whitney *U* test $P = 0.001$) [5]. Only 10% of patients with common HLA haplotypes waited longer than 3 months for a conclusion of the search process due to the prolonged donor activation time resulted, for example, from a withdrawal of a donor from the registry. In addition, we analyzed the presence of the progression in stage of the disease during the search process. It became apparent that proportions of patients who advance in stage of the disease were similar in patients with a short and a longer search process (median cut-point: 11% versus 14%, Chi-square test $P = 0.865$). This shows that in both groups there were patients with diseases at similar levels of relapse/progression potential. Time from the diagnosis to transplantation is influenced by several factors, including biology of underlying diseases and willingness of patients to undergo transplantation as an optional treatment. However, patients with a long time between the diagnosis and transplantation in the more homogeneous group of acute leukemias had more frequently rare alleles and/or B-C or DR-DQ associations than those being transplanted sooner after diagnosis (1-year cut-point: 50% versus 14%, Chi-square test $P = 0.035$). Therefore, length of the search process and the level of matching are major factors affecting post-HSCT survival. It enabled the development of a model predicting survival according to the level of matching and the time of the search process. Figures 1 and 2 show the predicted survival curves.

Table 3: Univariate analysis (continuous variables).

	HR	*P* value
Donor age (y)	1.004	0.775
Duration of the matching procedure (mth)	1.138	0.013

Table 4: Multivariate analysis.

	HR	*P* value
Donor-recipient HLA matching (mismatched)	2.422	0.007
Duration of the matching procedure (mth)	1.109	0.045

In addition we investigated whether time of the search procedure was affected by the number of matching attempts. It became apparent that more than two CT procedures resulted in a significant prolongation of the donor search completion (median: 1.5 versus 2.7 months, Mann-Whitney *U* test $P = 0.0002$; Figure 3).

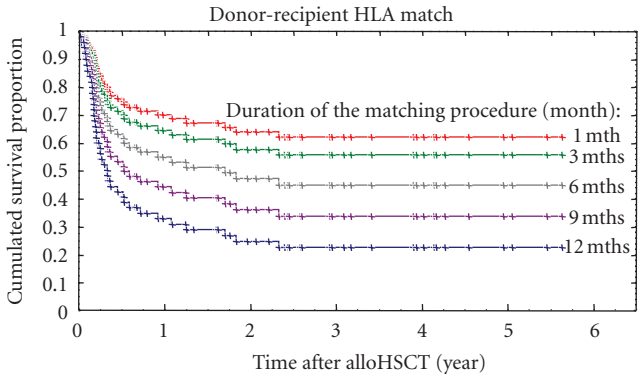

Figure 1: Survival curves of patients receiving alloHSCT from matched unrelated donor with respect to the duration of the matching procedure (as predicted according to the model).

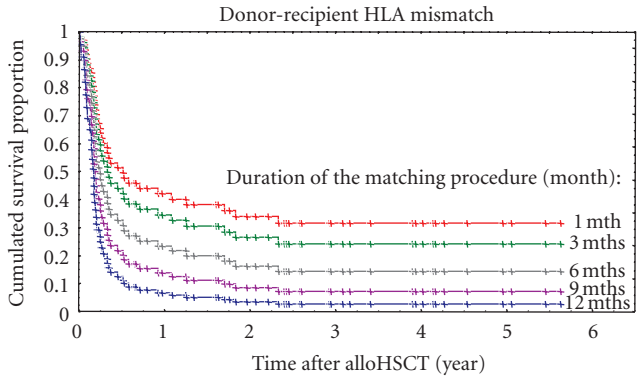

Figure 2: Survival curves of patients receiving alloHSCT from mismatched unrelated donor with respect to the duration of the matching procedure (as predicted according to the model).

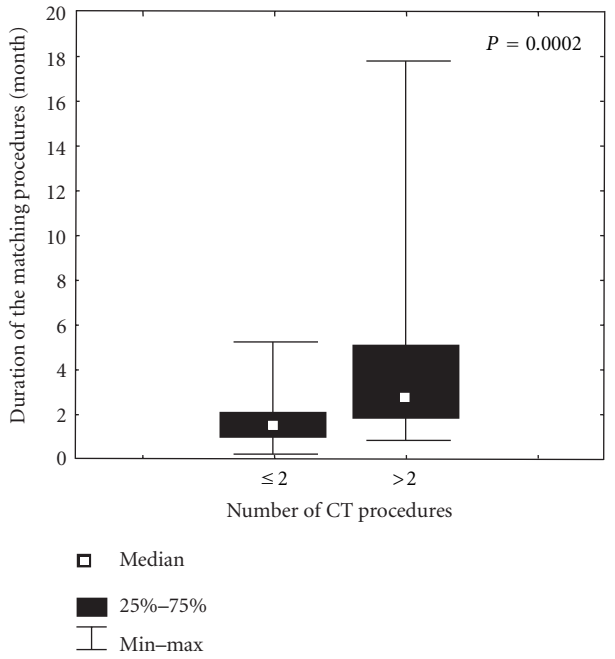

Figure 3: The duration of the matching procedure with respect to the number of CT procedures.

4. Conclusions

Data recorded in this study enabled us to confirm already known factors, namely, number of pregnancies and female-to-male donation, as those affecting survival after HSCT. This observation, concordant with other studies [9], shows that the donor-recipient pairs presented in this paper share similar characteristics with other reported HSCT groups of donors and recipients. Also it is apparent from the present study that the level of HLA matching plays an important role. This is also a well-known observation [10]. Keeping in mind the latter data, transplant centers frequently focus on the level of matching, neglecting the time needed for a prolonged procedure if the matching process is rather complex. Indeed, the time from the beginning until the completion of the search significantly depends on the number of confirmatory typing procedures performed. The novel aspect of the present paper is the finding that time needed for optimal match adversely affects the survival. Therefore, an optimal match reached after prolonged time results in a similar survival as that not optimal but completed promptly. Several previously published studies suggested ways to predict the length of the process on the basis of the HLA specificity profile in patients. This was also shown in the present group as patients with HLA rarities waited longer. Tiercy et al. [4] documented poorer survival in patients with rare alleles and B-C or DR-DQ associations. In the present study survival was analyzed not according to the HLA specificities associated with prediction but independently of any specific factors; just length of the search process was taken as a variable. Indeed, HLA rarities play an important role, but also other factors may be associated. Ten percent of patients with rather common HLA specificities waited for the search conclusion longer than 3 months. The reason of such delay is not entirely clear, but withdrawal of a potential donor from the registry may serve as an example.

The present study offers a rationale for the observation in the paper by Heemskerk et al. [11] that to achieve transplant results in the range of sibling transplantations the search procedure should be similarly time consuming.

Acknowledgments

This work was supported by Grant no. N R13 0082 06 from the Polish Ministry of Science & Higher Education.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 180391, 13 pages
doi:10.1155/2012/180391

Review Article

NOD2 Polymorphisms and Their Impact on Haematopoietic Stem Cell Transplant Outcome

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Received 28 June 2012; Accepted 3 September 2012

Academic Editor: Andrzej Lange

Haematopoietic stem cell transplantation (HSCT) is a valuable tool in the treatment of many haematological disorders. Advances in understanding HLA matching have improved prognoses. However, many recipients of well-matched HSCT develop posttransplant complications, and survival is far from absolute. The pursuit of novel genetic factors that may impact on HSCT outcome has resulted in the publication of many articles on a multitude of genes. Three *NOD2* polymorphisms, identified as disease-associated variants in Crohn's disease, have recently been suggested as important candidate gene markers in the outcome of HSCT. It was originally postulated that as the clinical manifestation of inflammatory responses characteristic of several post-transplant complications was of notable similarity to those seen in Crohn's disease, it was possible that they shared a common cause. Since the publication of this first paper, numerous studies have attempted to replicate the results in different transplant settings. The data has varied considerably between studies, and as yet no consensus on the impact of *NOD2* SNPs on HSCT outcome has been achieved. Here, we will review the existing literature, summarise current theories as to why the data differs, and suggest possible mechanisms by which the SNPs affect HSCT outcome.

1. Introduction

Allogeneic haematopoietic stem cell transplantation (HSCT) is an important treatment option in the management of many diseases including malignant and non-malignant haematological disorders, immune deficiencies and inborn errors [1]. The increased knowledge of transplant biology and the effects of clinical factors and HLA matching have improved outcome. The primary choice of donor is usually an HLA-matched sibling, but the probability of a sibling being HLA identical is only 25%, a problem that is exacerbated due to small family sizes that are usually found today. Alternative allogeneic donor sources are thus often required and have now become an important and viable option. There are currently over 19.8 million volunteer unrelated donors (UDs) that have been recruited to registries around the world, with an additional 543,000 umbilical cord blood units also being available (as of September 2012) (http://www.bmdw.org/). The improvement in transplant techniques and practice has resulted in similar survival prospects for recipients of a well-matched UD as that using a sibling [2, 3]. However, the risk of posttransplant complications such as graft-versus-host disease (GvHD) and delayed immune reconstitution leading to infection is increased [4].

The vital role of HLA matching in transplant outcome is accepted, but there is still controversy as to which of the six major HLA genes are most important. The current perspective on what constitutes a well-matched donor is a 10/10 HLA allele match that is matched at an allele level for HLA-A, -B, -C, -DRB1, and -DQB1 [3, 5–7]. Comprehensive analyses of UD-HSCT pairs have shown that allelic mismatches are as detrimental to transplant outcome as antigenic mismatches, with a single allelic mismatch at HLA-A, -B, -C, or -DRB1 being associated with an increase in GvHD and a reduction in overall survival. This data has been

confirmed in increasingly larger cohorts [8–11]. Mismatches at HLA-DQB1 appear to be better tolerated in the context of an 8/8 HLA-matched background (that is matched for HLA-A, -B, -C, and -DRB1) although there is some suggestion that they have a cumulative effect with any other HLA mismatch [6, 9, 10].

While the current donor selection criteria for matching donors and recipients usually refer to five of the classical HLA genes (HLA-A, -B, -C, -DRB1, and -DQB1), the impact of a sixth gene, HLA-DPB1, on the outcome of UD-HSCT is emerging. Current data suggests that nonpermissive HLA-DPB1 mismatches increase the risk of GvHD and transplant-related mortality [12–15].

Despite the benefit resulting from having a 10/10-matched donor, the survival of such a group of individuals is far from being absolute. Recipients receiving a graft from a well-matched sibling donor can be susceptible to getting GvHD. Conversely, some recipients of ≤9/10 HLA-matched grafts do survive and can achieve full remission of their disease [16]. While clinical factors such as the type of disease, disease stage, and recipient/donor characteristics are most certainly involved, theories have evolved that postulate a role for genes other than HLA in predicting transplant outcome. In recent years, much interest has been shown in the role of SNPs within innate immune response genes on the outcome of HSCT [17, 18]. One of the most prolifically studied genes to date has been the nucleotide-binding oligomerisation domain containing 2 (*NOD2*) gene (previously known as the caspase recruitment domain, family member 15 (*CARD15*) gene). The data from these studies is conflicting. Here, we will review the current data, on the impact of *NOD2* polymorphisms on the outcome of HSCT, potential causes of differences in the data and possible mechanisms by which the variants affect outcome.

2. *NOD2* Gene Structure and Function

The *NOD2* gene is located in humans on chromosome 16 (16q21) [19]. It is approximately 36 kb in length (35,938 bp) and encodes a protein of 1040 amino acids. *NOD2* encodes the NOD2 protein, a member of the NLR (NOD, leucine-rich repeat (LRR) containing) protein family [20–22]. Other members of this family include apoptosis protease-activating factor-1 (Apaf-1) and the MHC class II transactivator (CIITA) [23]. These proteins are classified by their common tripartite domain structure, namely, a central nucleotide binding domain (NBD, the NOD molecule), an amino terminal effector-binding domain (EBD), and a carboxy-terminal ligand-recognition domain (LRD). While all members of this family contain the central NBD region, the EBD and LRD differ between the different proteins. In NOD2, the central NBD domain is an NOD molecule which is surrounded by two CARD molecules (the EBDs) which enable recruitment of downstream signalling molecules and a series of 11 leucine rich repeats (LRR) which function as the LRD [24–26].

Early functional studies identified NOD2 expression in antigen-presenting cells, specifically intestinal epithelial cells [27], Paneth cells [28, 29], macrophages, and dendritic cells [21]. An increasing number of studies have demonstrated that NOD2 is expressed in a multitude of tissues including keratinocytes [30], T cells [31], NK cells, and CD34+ bone marrow stem cells [32, 33]. NOD2 is expressed within the cytosol and can be recruited to the cell membrane of intestinal epithelial cells [34, 35], a mechanism that appears to be important in the function of the molecule. Proinflammatory cytokines have been shown to regulate NOD2 expression [36].

The NOD2 protein functions as a regulator of infection by the recognition of pathogenic ligands and the induction inflammatory responses via a number of pathways. The most studied interaction is the response to the bacterial ligand muramyl dipeptide (MDP), a derivative of peptidoglycan, which is a component of both Gram-positive and -negative bacterial cell walls [37, 38]. Recognition of MDP by the LRD of NOD2 initiates a complex change in the structure of the molecule, enabling it to undergo self-oligomerisation via the NBD [25, 26, 39], and subsequently the recruitment of the effector molecule receptor-interacting, CARD-containing serine/threonine kinase (RICK) via homophilic interaction of their CARD domains. This recruitment of RICK by NOD2 causes the effector molecule to be activated, and initiates the downstream signalling events that lead to the induction of the nuclear factor (NF)-κB and mitogen-activated protein kinase pathways [39–41]. In addition to this cytokine response initiated by bacterial infection, it has also been shown that upon exposure to MDP, NOD2 plays a key role in the initiation of the autophagy pathway [42, 43]. NOD2 has also been shown to respond *in vitro* to viral infection by the recognition of a single-stranded (ss) RNA ligand [44]. Here, ssRNA binds to the LRD of NOD2, but rather than recruiting the RICK as an effector molecule, NOD2 is translocated to the mitochondria where it is able to interact with the mitochondria antivirus signalling protein and initiates downstream signalling of the NF-κB pathway.

3. Genetic Polymorphism of the *NOD2* Gene

The *NOD2* gene is proving to be highly polymorphic with over 660 single nucleotide polymorphisms (SNPs) reported to date both in the literature [45–47] and in various online databases (http://www.genecards.org/, http://www.ensembl.org/ and http://fmf.igh.cnrs.fr/ISSAID/infevers/) [48–50]. The minor allele frequencies vary from less than 1% to over 30%, although significant differences between different ethnic and geographic populations have been demonstrated.

Early studies to identify possible genetic factors that were affecting the incidence of Crohn's disease, a chronic inflammatory disorder of the gastrointestinal tract that can be complicated by anaemia, stenosis, and fistulae, mapped *NOD2* as a susceptibility locus [19]. Further studies identified three polymorphisms (designated nomenclature: SNP 8 (reference SNP (rs) rs2066844), SNP 12 (rs2066845) and SNP 13 (rs41450053)) as disease-associated polymorphisms (Figure 1) [45, 51]. It has been shown that individuals heterozygous for any of the three SNPs have a two- to

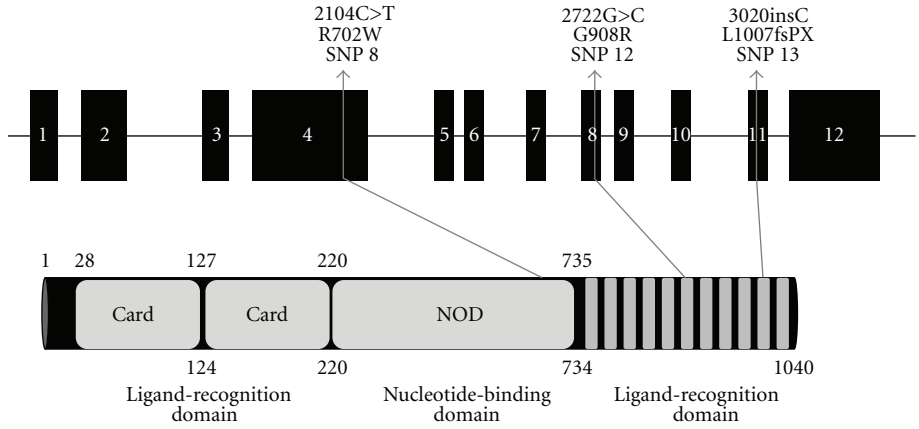

Figure 1: The structure of the *NOD2* gene and NOD2 protein. The numbering in the black boxes indicates the exon numbers. The numbering alongside the protein diagram indicates the amino acid positioning. SNPs 8, 12, and 13 are located within exons 4, 8 and 11 respectively, and encode either amino acid substitutions (SNPs 8 and 12) or a frame-shift causing early truncation of the protein (SNP 13).

fourfold increase in the risk of developing Crohn's disease, which increases to approximately twentyfold in individuals who are homozygotes or compound heterozygotes [52]. Other disease-associated studies have also tried to identify the impact of these three polymorphisms with varying results [53]. Subsequently, SNPs 8, 12, and 13 have become some of the most studied and well-characterised SNPs of the *NOD2* gene.

SNPs 8, 12, and 13 are located within *NOD2* exons 4, 8 and 11 respectively. SNPs 8 and 12 are nonsynonymous nucleotide substitutions that result in amino acid changes, SNP 8 (coding (c.) 2104C>T, protein (p.) R702W) and SNP 12 (c. 2722G>C, p. G908R). SNP 13 differs in that it involves the insertion of a nucleotide that results in a frameshift within the coding sequence causing the introduction of an early termination codon and thus a truncated protein (c. 3020CinsC, p. L1007fsPX). SNP 8 is located within the central NBD region of the molecule, while SNPs 12 and 13 are found within LRRs 7 and 10, respectively, of the NOD2 LRD [25, 46].

4. *NOD2* Gene Polymorphisms and Disease

Following the early studies in Crohn's disease, polymorphisms throughout the *NOD2* gene have been implicated in numerous diseases. SNPs 8, 12, and 13 have been correlated with increased risk of ankylosing spondylitis [54], psoriatic arthritis [55], and more recently with early-onset sarcoidosis [56]. Three additional polymorphisms, p. R334W, p. R334Q, and p. L469F, have been associated with Blau syndrome [57]. In addition to these inflammatory disorders, *NOD2* SNPs 8, 12, and 13 have also been correlated with an increased risk of malignant diseases such as colorectal [58], gastric [59], breast, and lung cancer [60] as well with the incidence of non-hodgkin's lymphoma [61], although in most of these studies, the detrimental effects of *NOD2* genotype were limited to the presence of SNP 13. More recently, *NOD2* SNPs have been shown to affect graft survival and mortality post renal transplantation [62] and coronary artery disease [63].

5. The Functional Consequences of *NOD2* SNPs 8, 12, and 13

SNPs 8, 12, and 13 are thought to reduce the ability of MDP to activate NOD2 and consequently the activation of NF-κB, resulting in reduction in the production of cytokines and antimicrobial peptides [64–66]. These loss-of-function effects caused by the SNPs initially proved controversial, as an enhanced cytokine response is characteristic of Crohn's disease. The publication of data that showed mice with an *NOD2* variant similar to SNP 13 had increased sensitivity to MDP and elevated levels of NF-κB activation when compared to WT mice suggested a gain-of-function effect of *NOD2* SNPs [67, 68]. While this evidence showed a plausible mechanism by which *NOD2* variants contributed to the onset of Crohn's disease, these findings have not been replicated in human studies, and further data has been published that confirm the loss-of-function mechanism [69–72]. Thus, the *NOD2* variants appear to reduce the ability of NOD2 to recognise MDP and consequently to stimulate NF-κB responses. It has been suggested that the inflammatory response seen in Crohn's disease results from the inability of toll-like receptor-2 (TLR-2) to become tolerant to its ligand in the absence of appropriately functioning NOD2, resulting in upregulation of proinflammatory cytokines [73, 74]. In addition to these effects, SNPs 8, 12, and 13 have been associated with increased permeability of the gastrointestinal mucosa and consequently increased levels of bacterial peptides in systemic circulation [75].

The impact of *NOD2* variants other than the three aforementioned SNPs has not been investigated to the same extent. *NOD2* polymorphisms outside of the LRD do not appear to alter the ability of MDP to stimulate NOD2. In the case of the variants associated with Blau syndrome, all of which are located within the central NOD region of

the protein, an increase in NF-κB activity has been reported [25, 65]. This gain-of-function mechanism appears to be consistently demonstrated.

6. *NOD2* Polymorphism and the Outcome of HSCT

It was originally postulated that the *NOD2* variants that are purported to increase the risk and severity of Crohn's disease might also contribute to the risk of GvHD, particularly gastrointestinal GvHD, due to their notable similarity in clinical symptoms [76]. In the years following, many groups have published data on their attempts to test this hypothesis in a number of different transplant settings. Table 1 summarises the differences in the cohort characteristics and the clinical observations reported by each group.

In the first published study by Holler et al. [76] 169 HSCT pairs underwent *NOD2* genotyping for SNPs 8, 12 and 13. The cohort consisted of a mix of HLA-matched related donor, unrelated donor and a small number of one HLA antigen-mismatched related donor, transplants. Transplants were performed as a therapy for acute leukaemia, myeloproliferative disorder, lymphoma, or myeloma. Approximately 44% of the cohort underwent T-cell depletion, predominantly with antithymocyte globulin (ATG), while a small number of individuals were treated with alemtuzumab or CD34+ cell selection. The results of this study showed that 29.5% of HSCT pairs in this cohort had at least one of the *NOD2* variants. The authors correlated the presence of any of the three SNPs in the genotype of the pair (recipient, donor or both SNP positive) with increased severe aGvHD, (grades III-IV), severe gastrointestinal aGvHD and nonrelapse mortality [76]. When this was broken down further, severe aGvHD was increased in pairs with SNP-positive donors only, while an increase in severe and gastrointestinal aGvHD was described in pairs where both the recipient and donor were found to have any of the variants. This consequently increased the risk of nonrelapse mortality.

In their subsequent analysis, the authors extended the cohort to 303 HLA-matched sibling HSCT pairs, transplanted at one of five European centres [77]. The underlying disease of the recipients was acute leukaemia, chronic leukaemia, bone marrow failure syndromes, or lymphatic malignancies. The authors did not report the use of T-cell depletion. *NOD2* genotyping of recipients and donors showed similar frequencies of SNPs 8, 12, and 13 to their earlier study and, importantly, between the different cohorts that were included in the study. The data showed that the effect of *NOD2* variants on clinically significant aGvHD (grades III-IV) and gastrointestinal GvHD persisted in this new cohort, while a trend for increased cGvHD was also noted. A dosage effect of the SNPs was seen in this study where individuals with increasing numbers of SNPs correspondingly had an increasing risk of aGvHD. The SNP dosage effect was also seen on the incidence of nonrelapse mortality. Survival was affected, but only when variants were present in the recipient genotype or in both the recipient and donor genotypes. The authors also described how the use of particular gastrointestinal decontamination agents could reduce the risk of aGvHD and nonrelapse mortality seen with *NOD2* SNPs. Specifically, the effects of *NOD2* variants were only seen in individuals who received either no decontamination or those whose protocol included the antibiotic Ciprofloxacin.

In their third and most recent study, Holler and colleagues have extended their cohorts further to include 358 HLA matched related donor and 342 unrelated donor HSCT pairs [78]. Approximately 55% of the cohort underwent HSCT for acute leukaemia. The use of T-cell depletion varied between the two subgroups that made up the cohort, with 78% of cohort one (HSCT pairs from earlier studies) having some form of T-cell depletion included as compared to only 22% of cohort two (additional HSCT pairs). The impact of *NOD2* variant genotype was analysed separately in the related and unrelated donor cohorts. The presence of any *NOD2* variant in the genotype of the pair was correlated with significantly increased severe aGvHD (grades III-IV), nonrelapse mortality and reduced overall survival in recipients of a related donor HSCT. In the UD-HSCT cohort, aGvHD was the only outcome affected by the presence of any of the three SNPs, while detrimental effects on nonrelapse mortality and survival were associated with the presence of SNP 13 within the donor's genotype. The association of specific gastrointestinal decontamination protocols (either none or Ciprofloxacin-based therapies) with increased effects of *NOD2* variants was confirmed in these cohorts.

Other groups have confirmed the effects of *NOD2* variant genotype on HSCT outcome described by Holler et al. A recent study by a group in The Netherlands described the effects of *NOD2* SNPs 8, 12, and 13 on the outcome of 85 HLA-identical sibling transplants [79]. The cohort included recipients with acute leukaemia, chronic myeloid leukaemia, myeloproliferative disorder, myelodysplastic syndrome, and lymphoma. The entire cohort had a partial T-cell depletion protocol included in their transplant protocols with the most common method being CD34+ cell selection. *NOD2* variant frequencies were similar to those reported in the earlier studies and in the general Dutch population. The authors confirm the detrimental effect of any *NOD2* variant on the risk of clinically significant aGvHD and nonrelapse mortality. As described in the earlier studies, the effect was most profound when both the recipient and donor were positive for any one of the SNPs.

Not all studies have been able to demonstrate an association of *NOD2* polymorphisms with GvHD. Elmaagacli and colleagues published data on the effect of the variants in a cohort of 403 related and unrelated donor transplants [80]. The recipients were transplanted for numerous diseases, predominantly acute leukaemia, chronic myeloid leukaemia, and myelodysplastic syndrome. Approximately 30% of the cohort had T-cell depletion included in the conditioning regimens either with alemtuzumab or with ATG. The frequency of *NOD2* variants in this cohort was similar to those described in other studies. Although an increased risk of aGvHD (grade III-IV) was seen when recipients and donors were both positive for one of the *NOD2* variants, a protective effect was associated with an SNP in the donor

Table 1: A comparison of the results published on *NOD2* genotype and haematopoietic stem cell transplant outcome.

Study	Donor source[a]	Recipient diagnosis[b]	T-cell depletion	Effect of *NOD2* SNPs
Holler et al. 2004 [76]	Mixed	Mixed	Yes—*in vivo* 43% WT pairs 48% SNP pairs	Increased severe aGvHD (gr. III-IV) in SNP-positive donors and pairs Increased severe GI aGvHD with SNP-positive pairs; Increased transplant-related mortality in SNP-positive pairs
Holler et al. 2006 [77]	RD	Mixed	—	Reduced overall survival with SNP-positive recipients and when both recipient and donor are SNP positive Increased transplant-related mortality with increasing numbers of SNPs Increased severe (gr. III-IV) and severe GI aGvHD with increasing numbers of SNPs
Elmaagacli et al. 2006 [78]	Mixed	Mixed	Yes ~30%	Lower overall and severe aGvHD (gr. III-IV) with SNP-positive donors Increased severe aGvHD (gr. III-IV) when both recipient and donor are SNP positive Reduced disease relapse when both recipient and donor are SNP-positive
Granell et al. 2006 [81]	RD	Mixed	Yes, 100%	Reduced disease-free survival in SNP positive recipients.
Mayor et al. 2007 [82]	UD	Acute leukaemia	Yes—*in vivo* Alemtuzumab 82% WT pairs 85% SNP pairs	Reduced overall survival in SNP-positive recipients and pairs Increased disease relapse in SNP-positive recipients and pairs Reduced disease-free survival in SNP-positive recipients and pairs
Holler et al. 2008 [78, 83]	Mixed	Mixed	Yes, 78% cohort 1, 22% cohort 2	Reduced overall survival in SNP-positive pairs (sibling HSCT) and SNP 13 positive donors (UD) Increased severe aGvHD (gr III-IV) with SNP-positive pairs (sibling and UD) and SNP 13 positive donors (UD) Increased transplant-related mortality with SNP-positive pairs (sibling HSCT) and SNP 13 positive donors (UD)
van der Velden et al. 2009 [79]	RD	Mixed	Yes, 100%	Increased severe aGvHD (gr. III-IV) in SNP-positive pairs Increased transplant-related mortality in SNP-positive pairs
Wermke et al. 2010 [84]	Mixed	Mixed	Yes, *in vivo* ATG for UD, 31.6%	Increased disease relapse in SNP-positive recipients (trend at MV) Trend for less GI aGvHD in SNP-positive recipients (UV only)
Elmaagacli et al. 2011 [85]	RD	AML	None	Increase in overall (gr. I–IV) and severe (gr. III-IV) aGvHD in SNP-positive recipients
Kreyenberg et al. 2011 [86]	Unknown allogeneic	Mixed	—	Reduced overall survival in SNP 13 positive recipients Increased transplant-related mortality in SNP 13 positive recipients
Ditschkowski et al. 2007 [87]	Mixed	Mixed	Yes, 30% *in vivo*	No significant effects on BO or BOOP
Hildebrandt et al. 2008 [88]	Mixed	Mixed	Yes, 37%	BO increased in SNP-positive recipients
Sairafi et al. 2008 [89]	Mixed	Mixed	Yes, 61%	No significant effects
Gruhn et al. 2009 [90]	Mixed	Mixed	None	No significant effects
Nguyen et al. 2010 [91]	UD	Mixed	None	No significant effects
van der Straaten et al. 2011 [92]	Mixed	Mixed	Yes, *in vivo* ATG for UD and HLA-mismatched RD	No significant effects

[a] Mixed as a donor source denotes both related (RD) and unrelated donors (UD) were used.
[b] Mixed as a recipient diagnosis indicates that the included recipients underwent HSCT for any one of a number of diseases.

genotype. The protective effect was also seen on disease relapse in pairs where both the recipient and donor had at least one *NOD2* SNP. Unlike previous studies, no effects of *NOD2* genotype on nonrelapse mortality or survival were seen. The authors suggested that the possible reason for the lack of association here was due to their routine use of gastrointestinal decontamination with agents to target both Gram-positive and negative bacteria.

In a recent update by this group, the authors have investigated the affects of *NOD2* variants in a more homogeneous cohort [85]. *NOD2* genotyping was performed on a cohort of 142 AML recipients and their HLA-matched sibling donors. As in previous studies, the reported frequency of SNP-positive recipients and donors was similar to those found elsewhere. The cohort only included recipients who received myeloablative conditioning regimens and T-cell replete grafts. Unlike in their previous study, no protective effects of *NOD2* SNPs were associated with GvHD. A significant association was seen between SNP-positive recipients and an increased risk of any aGvHD (grade I–IV) and severe aGvHD (grades III-IV). Interestingly, after multivariate analysis, only a correlation with grade II–IV remained significant (relative risk (RR) 3.7652, $P < 0.002$). No impact on overall survival or nonrelapse mortality was reported.

Granell et al. also failed to correlate *NOD2* genotype with increased aGvHD [81]. Here, *NOD2* genotyping was performed on 85 HLA-matched sibling HSCT pairs. The underlying diseases of the recipients were acute leukaemia, myeloproliferative disorder, lymphoma, myeloma, myelodysplasia, aplasia, and chronic lymphocytic leukaemia. All recipients had T cell depletion included in their conditioning regimens, although the method was not reported. The authors report an association of recipient *NOD2* variant genotype with significantly reduced event-free survival. No other variable was significantly affected [81].

Our group has also reported the effects of *NOD2* genotype on HSCT outcome [82]. Here, the impact of *NOD2* genotype was investigated in a cohort of 196 recipients of an unrelated donor HSCT for an acute leukaemia. T-cell depletion was included in the conditioning regimens of 83% of recipients, with *in vivo* alemtuzumab being the preferred method. We reported a significant correlation between SNP-positive pairs (the recipient, the donor, or both had any *NOD2* SNP) and increased risks of disease relapse and death. In accordance with the data published by Granell et al. [81], we were also able to show a significant association with event-free survival. Interestingly, although the overall incidence of aGvHD was low in this British cohort due to the near universal use of T-cell depletion, a protective effect of *NOD2* SNPs on aGvHD was noted although it remained nonsignificant. Despite failing to achieve statistical significance, this data was in accordance to that reported by Elmaagacli and colleagues [80].

A study published in 2010 from a group in Dresden, Germany also reported a correlation between *NOD2* genotype and disease relapse [84]. This single-centre study included 304 HSCT pairs where the predominant diagnoses were AML/MDS (52%) and lymphoma (25.3%). Grafts were from either a ≥8/10 HLA matched unrelated donor (67.1%) or an HLA-matched related donor. Recipients receiving a graft from an UD had *in vivo* ATG included in their conditioning regimens. The authors performed extensive analyses to determine if an association between *NOD2* genotype and aGvHD could be identified. A trend towards reduced gastrointestinal aGvHD was reported in recipients positive for any *NOD2* variant, but this affect was limited to univariate analyses. There were no significant differences in GvHD in any of the other models tested. Recipients positive for any of the three SNPs did have a significantly increased risk of disease relapse, although this was only a trend after multivariate analysis ($P = 0.056$).

A brief communication published last year highlighted the impact of *NOD2* SNPs in a large, multicentre, paediatric cohort [86]. A total of 567 HSCT pairs were tested. Donors were both HLA matched (78.7%) and mismatched (21.3%); the type of allogeneic donor was not stated. Transplants were performed for haematological malignancies, nonhaematological malignancies, and nonmalignant disease. The authors describe a significantly increased risk of nonrelapse mortality in recipients positive for SNP 13, an effect that persisted after multivariate analysis (RR 2.01, $P = 0.049$). This study also confirmed the effects of *NOD2* genotype on overall survival. A trend for lower survival was reported in pairs where the recipient had at least one of the three variants. Additionally, survival was also lower in recipients only positive for *NOD2* SNP 13.

Two studies have specifically reported data on the impact of *NOD2* variants on bronchiolitis obliterans (BO) and bronchiolitis obliterans organising Pneumonia (BOOP), two serious late-onset, non-infectious pulmonary complications that can occur after HSCT. Hildebrandt et al. [88] analysed the incidence of BO/BOOP in a heterogeneous cohort of 427 HSCT pairs. Donors were either HLA-matched siblings or UDs. T cell depletion was included in the conditioning protocols of approximately 25% of the cohort although the method varied (ATG, alemtuzumab, or CD34+ selection). The incidence of BO was significantly higher when recipients, donors, or both were positive for *NOD2* SNPs, effects that persisted after multivariate analysis. It is important to point out, however, that the overall number of recipients who developed BO was very low in this cohort (11/427, 2.6%). In contrast to this data, Ditschkowski et al. did not find an association between *NOD2* genotype and the incidence of BO/BOOP in their cohort of 281 sibling donor HSCT pairs [87]. Transplants were for acute and chronic leukaemia, myelodysplastic syndrome, non-Hodgkin's lymphoma, idiopathic mnyelofibrosis, and multiple myeloma, and approximately 30% protocols included *in vivo* T cell depletion. As in the previously described study, the overall incidence of BO/BOOP was low (2.1% BO, 3.6% BOOP).

Despite the plethora of data available showing an effect of *NOD2* variants, several studies have suggested that there are no significant effects on HSCT outcome. Groups from Sweden [89], Germany [90], the United States [91] and The Netherlands [92] have performed extensive analyses in attempt to replicate the findings of the above-mentioned studies but have shown a lack of association with any of the outcomes measured.

7. Discussion

There does not yet appear to be a consensus on the impact of *NOD2* variants on the outcome of HSCT. It would be reasonable to assume that the potential mechanisms of how the SNPs cause functional irregularities may be common but that the manifestation of the effects differs between groups. Here, we will discuss possible mechanisms by which *NOD2* genotype may affect HSCT outcome.

NOD2 is known to function as a regulator of cytokine production and a mediator of proinflammatory responses upon recognition of the bacterial ligand muramyl dipeptide [40, 93]. Functional changes within the NOD2 protein are seen with SNPs 8, 12, and 13, all resulting in down regulation of cytokine production via the NF-κB pathway [33, 94]. This dysregulation of cytokine production may provide the first mechanism by which *NOD2* variants can affect the outcome of HSCT.

An early event posttransplant is the onset of the "cytokine storm" [95], an extreme increase in cytokine production as a response to both tissue damage in the recipient resulting from conditioning regimens and the activation of donor derived T cells to recipient alloantigens [96]. The result of the cytokine storm is the onset of both GvHD and graft-versus-leukaemia (GvL) responses [97, 98]. These tumour-specific cells are thought to be of T cell origin but data is emerging that suggest other cell types such as NK [99] and NKT cells [100] are also involved. One possible explanation of how *NOD2* genotype causes an effect after HSCT is that the inability of the NOD2 variant proteins to initiate cytokine production could, in theory, lead to a massive disruption of the cytokine storm, resulting in a lack of GvL or GvHD responses.

While the effect of *NOD2* genotype-related dysregulation of cytokine production may not be the only contributing pathway to the cytokine storm, the role of NOD2 and other sensors of bacterial infection has long been proposed as major factors in GvHD responses. Studies that have shown that gastrointestinal mucosa damaged by aggressive treatments such as the conditioning regimens used in HSCT allow bacterial ligands, specifically the MDP homologue Lipopolysaccharide (LPS), to seep into systemic circulation. Once there, T cells specific for these ligands are capable of stimulating cytokine production and eliciting GvHD responses [101–103]. It has been suggested that *NOD2* SNPs can increase the permeability of the gastrointestinal mucosa and potentially increase the ability of bacterial ligands to enter systemic circulation [75]. It is possible that these events in combination with the inability of the variant NOD2 protein to respond efficiently to bacterial infection in recipients with *NOD2* variant genotype result in an increased level of circulating LPS, which are able to prime T cells and thus initiate strong GvHD responses. These effects are in concordance with the data published by numerous groups correlating *NOD2* variant genotype and increased aGvHD.

NOD2 is also known to have a synergistic relationship with TLRs and is thought to provide some regulatory control over their ability to stimulate cytokine production [93, 104–106]. It is possible that the inability of variant NOD2 to regulate or be regulated by TLRs resulted in dysregulation of the cytokine produced, which in turn affected both GvHD and GvL responses. One of the most studied relationships is with TLR2 [107]. NOD2 is known to act as a regulator of IL-12 production via the simultaneous stimulation of NOD2 and TLR2 by their bacterial ligands with both positive and negative regulation occurring dependant on the dose of available ligand [104, 107]. Polymorphisms of *NOD2* are known to cause a reduction in IL-12 production [69]. Interestingly, in the context of HSCT, low IL-12 levels have been correlated with an increase in the incidence of disease relapse [108] without increasing the incidence of aGvHD [108, 109].

NOD2 is expressed both intracellularly and on the cell surface of epithelial cells. It has been suggested that this membrane recruitment of the protein is necessary to initiate a functional response [34, 35]. The repertoire of known cell types showing NOD2 expression is increasing, with both NK cells and CD34+ bone marrow stem cells recently being identified [32, 33]. It is thus feasible to assume that NOD2 is expressed on the cell surface of these other cell types. The presence of SNP 13 has been associated with the failure of the molecule to be expressed on the cell surface, although this has not been reported for the other polymorphisms [34, 35]. It is possible that the failure of leukaemic cells to express NOD2 extracellularly in recipients with *NOD2* variant genotypes results in their evasion of immunesurveillance activity. This escape mechanism would lead to the proliferation of leukaemic cells and thus disease relapse after transplant. This theory is consistent with the observations that *NOD2* polymorphisms cause disruption of GvL responses.

Although no effect of *NOD2* SNPs 8 and 12 on the membrane recruitment of NOD2 has been reported to date, it is possible that they have an alternative mechanism by which they cause cells to evade immune responses. SNP 8 is located within exon 4 of the *NOD2* gene and is found between the NBD and the LRD of the protein [25, 110]. Self-oligomerisation of the protein occurs at the NBD, a process that is fundamental to the ability of the NOD2 protein to function [25, 111]. It is possible that SNP 8 causes a conformational change in the molecule rendering it either incapable of self-binding or causing it to function at a reduced capacity. Alternatively it may render the LRD either unable to or inefficient at binding its ligand. If this is the case, then it is feasible that even if NOD2 is recruited to the cell surface, it is unlikely to initiate a functional response that is adequate to initiate GvL effects. SNP 12 is located within the sixth LRR, which makes up the LRD [110]. The change in protein at this position may alter the ability of the *NOD2* molecule to recognise MDP, leading to the failure of NOD2 to initiate NF-κB signalling and its related downstream events.

A logical explanation for the divergent results could be the heterogeneity in the characteristics and treatment of the recipients, not only between studies but also within each of the cohorts themselves. An obvious difference between the studies is donor source. The advances in transplant techniques and practice have resulted in similar survival

prospects for recipients of a well-matched UD and related donor HSCT [2], suggesting that while donor source may contribute to the discrepancies in outcome associations reported, it is more likely that other characteristics of the cohort are correlated with outcome.

A second and strikingly different factor between the cohorts is the use of T-cell depletion within the conditioning regimens. T-cell depletion is used as a mechanism of reducing the risk of GvHD, although a consequence of this may be an increase in disease relapse [98, 112]. While most of the *NOD2* SNP association studies reported the use of T-cell depletion in their treatment protocols, several methods (alemtuzumab, ATG and/or CD34+ stem cell selection) were included, and thus it is important to consider the effectiveness of these different methods. For example, the anti-CD52 antibody alemtuzumab targets all human cells of lymphoid lineage, although NK cells appear to be relatively spared [113–115]. CD34+ stem cells are not targeted. Conversely, ATG functions by only targeting cell surface markers including those found specifically on T cells. B and NK cells are also targeted but only in excessive doses of ATG and are thus spared in most transplant protocols [116]. The effects of ATG are also long lasting which results in the specific depletion of T cells from the graft and any reconstituting cells. It is possible that the residual haematopoietic cells or indeed the lack of certain cell types present after different types of T-cell depletion could significantly affect the type and risk of post-transplant complication.

In addition to the method of T-cell depletion used, notable differences in the number of recipients treated varied between the studies (approximately 30–100%). It is interesting to note that a high number of studies that reported a correlation between *NOD2* genotype and GvHD were either T-cell replete regimens or included ATG or partial CD34+ cell-selected grafts [76, 78, 79, 85]. Conversely, those studies that correlated *NOD2* variants with impaired Graft-versus-leukaemia (GvL) effects included consistently higher numbers of recipients treated with T-cell-depleted protocols (85–100%) and in some cases included alemtuzumab [81, 82, 84].

Gastrointestinal decontamination, a method of using drugs to control levels of bacteria within the gastrointestinal tracts, may also be used all around transplantation as a method of controlling GvHD [103, 117]. Holler and colleagues have suggested that the impact of *NOD2* SNPs may be more evident in recipients who received either no decontamination or those who were treated with Ciprofloxacin-based therapy [77, 78]. Elmaagacli et al. (2006) suggested that the lack of correlation between their data and that previously published could be attributed to their universal use of a decontamination protocol that includes a second antibiotic, Metronidazole, in combination with Ciprofloxacin [80]. In addition, the study by van der Velden et al. also highlighted the important role of bacteraemia in the outcome of HSCT in their study [79]. Unfortunately, most of the studies published to date have not included data on the use and/or type of gastrointestinal decontamination in their cohorts, and a few have analysed the effects of *NOD2* variants in cohorts stratified by protocol. It would be prudent for future studies to include this data in their analyses where possible in order for the exact relevance of this information to be obtained.

Several studies, including ours, have demonstrated the effects of *NOD2* genotype in recipients diagnosed with an acute leukaemia [82, 83, 85]. We have also reported on the lack of effect in recipients with chronic myeloid leukaemia in our cohort from the UK [118]. Other studies have not fully investigated the suggestion of a disease-specific effect. However, it is interesting that two of the four studies that did not correlate *NOD2* genotype with any posttransplant complication had a notably low number of recipients with acute leukaemia in their analyses [91, 92]. A possible explanation for this apparent disease specific effect is that *NOD2* SNPs alter the responsiveness of recipients with an acute leukaemia to their treatment. This may occur by modulation of the pathways of disease progression, rendering recipients resistant to treatment. While no direct evidence of the involvement of NOD2 variants in leukaemia progression exists, there is much data to show how it can affect the other diseases that are associated with the polymorphisms. In Crohn's disease, *NOD2* SNPs 8, 12, and 13 have been correlated with distinct disease phenotypes, in particular with the site of Crohn's disease within the gastrointestinal tract and with the age of onset [119–122]. *NOD2* genotype may also alter the recipient's response to drugs or conditioning therapies. Studies have shown that *NOD2* polymorphisms can affect the response to antibiotic treatment of perianal fistulas in Crohn's disease patients. The data showed that patients with an *NOD2* WT genotype had a 33% rate of complete response to treatment as compared to none of the patients with *NOD2* variant genotypes [77].

While the majority of studies have shown an effect of *NOD2* genotype on transplant outcome, data has been published that contradicts these findings [89–92]. As discussed, the lack of effect could be attributed to several characteristics of the cohort, namely the graft source, type of disease, use and method of T-cell depletion, and gastrointestinal decontamination. However, a notable difference between several of these studies and others published is the low incidence of *NOD2* SNPs reported. The overall SNP frequencies were between 10–15% lower than reported elsewhere. The difference in the frequency of *NOD2* SNPs between different ethnic and geographic populations has been widely discussed [123–127]. Thus, the low prevalence of SNPs in these cohorts may mask any affects that the genotype is having on transplant outcome.

A common feature of many of the studies is the correlation between recipient *NOD2* genotype and detrimental posttransplant outcomes. This may imply that cells which express NOD2 and remain in the recipient after their conditioning regimens, such as tissue macrophages, dendritic cells, and Paneth cells, may facilitate GvHD or GvL responses, and that these responses are limited in recipients with *NOD2* variant genotypes. The ability of recipient cells, specifically dendritic cells, to initiate GvHD effects has been reported [128]. Additionally, recently published data has demonstrated the importance of recipient *NOD2* genotype in murine models of GvHD [129]. Here, murine recipients of bone marrow and/or T cells from either wild-type (WT)

or *NOD2* knock-out mice showed no significant differences in the ability of the repopulating cells to proliferate, to be activated, or on their expression of gut-homing molecules. The risk of developing GvHD was similar in the two groups. Conversely, *NOD2* knock-out recipient mice showed significantly higher levels of GvHD than their WT counterparts, and importantly, the organs targeted were the liver and the small and large bowels. Further tests showed that recipient *NOD2* genotype was also able to effect donor T-cell functional capabilities. While the translation of murine studies into human models does not always result in the same findings, these data in combination provide some evidence to substantiate the observation that recipient genotype appears to significantly correlate with HSCT outcome in humans.

The studies that have suggested the *NOD2* genotype results in impaired GvL responses do not fit this model. A possible explanation for this is that recipient cells that are more resistant to the effects of pretransplant conditioning regimens (in these studies, T-cell depletion in particular) are responsible for the lack of GvL effects. NK cells have been shown to be more resistant to the T-cell depletion agent alemtuzumab than other targeted subgroups [115]. The importance of NK cells in this model has been previously suggested [83], and their ability to function as tumour surveillance cells and mediators of antileukaemic responses is widely accepted [100, 130]. Importantly, it has been suggested that autologous NK cells can maintain remission in acute leukaemia patients, although this was described in the context of autologous transplants or chemotherapy induced remission [131]. NK cells have recently been shown to express NOD2 and also to be activated by the recognition of MDP by NOD2 in the presence of costimulatory molecules [32]. It is possible that this mechanism for NK cell activation is of critical importance in mediating early GvL responses after HSCT, but in recipients with *NOD2* variant genotypes, this NK cell activation is limited, resulting in a reduced ability to initiate GvL responses. Interestingly, in our study, where predominant T-cell depletion with alemtuzumab was used, an increase in disease relapse was seen in recipients with *NOD2* polymorphisms.

Finally, it is important to consider what impact *NOD2* polymorphisms other than SNPs 8, 12, and 13 may have on HSCT outcome. It is possible that these SNPs are only markers for detrimental outcomes and that the true association is with one or more untested polymorphisms that may be in linkage disequilibrium with these known variants. As stated previously, *NOD2* is highly polymorphic with some minor allele frequencies reaching 40% in certain populations. It would be prudent for future studies to consider the effects of the previously unstudied variants in any future analyses. It is possible that reanalysis of the published data including novel variants may result in concordance between different groups and potentially elicit an effect of *NOD2* genotype in cohorts where no association has been demonstrated previously.

Despite the many questions that remain even after eight years of investigation into the importance of *NOD2* genotype on HSCT outcome, it must be concluded that the gene and its variants currently indicate an important role in transplant biology. The published data also reaffirms the belief that personalised medicine based on a combination of recipient and donor characteristics, HLA matching, and non-HLA genetics could provide the key to superior outcomes after HSCT.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 485160, 9 pages
doi:10.1155/2012/485160

Review Article

The Role of HLA in Cord Blood Transplantation

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Received 23 July 2012; Accepted 12 September 2012

Academic Editor: Andrzej Lange

In recent years, umbilical cord blood (CB), a rich source of hematopoietic stem cells (HSC), has been used successfully as an alternative HSC source to treat a variety of hematologic, immunologic, genetic, and oncologic disorders. CB has several advantages, including prompt availability of the transplant, decrease of graft versus host disease (GVHD) and better long-term immune recovery, resulting in a similar long-term survival. Studies have shown that some degree of HLA mismatches is acceptable. This review is intended to outline the main aspects of HLA matching in different settings (related, pediatric, adult, or double-unit HSCT), its effect on transplantation outcome and the role of HLA in donor selection.

1. Introduction

The experience of the last 20 years indicates that cord blood transplantation is a valid alternative to bone marrow (BM) and PBSC transplants. For patients suffering from malignant or nonmalignant diseases, who do not have a matched sibling donor or a matched volunteer unrelated donor, two available alternative stem cell donor sources exist: a haploidentical transplantation from a three locus mismatched family member (parents, siblings) or an unrelated cryopreserved umbilical cord blood (CB) unit from a cord blood bank [1–4]. A low rate of graft versus host disease (GVHD) in the presence of higher HLA disparity, represents the main advantage of the umbilical cord grafts, while delayed engraftment due to limited cell dose is still the major drawback [3]. Moreover, umbilical cord blood is a viable source particularly for racial and ethnic minority patients whose genetic variations are not included in unrelated volunteer donor registries [5].

The role of HLA mismatches in CBT remains unclear as most transplants have been selected on low resolution class I HLA typing and allelic level class II typing. In malignant diseases, HLA mismatching is partially overcome by increasing the cell dose [6]. Recent data on associations between HLA disparity and survival, support that there is a direct association between the number of donor-recipient HLA mismatches and the risk for GVHD, while the mismatching has a greater impact on absolute mortality differences in recipients with diseases with low risk of posttransplant recurrence [7].

The number of CB transplantations, as well as the global inventory of CB units, are growing rapidly. CB grafts, in contrast to adults unrelated donors who need 10/10 allele level matches with the patients, have a reduced risk of severe GVHD and permit a mismatched transplantation at least in one HLA locus [8, 9]. HLA matching for unrelated CBT generally focuses on three HLA loci HLA-A,-B, and -DRB1. In order to overcome limitation in cell dose, many centers perform double unit CBT (dCBT) [10].

This paper focuses on the impact of HLA-matching in CBT in different settings: related, unrelated, pediatric, adult, and double CBT; the eventual inclusion of other HLA loci in the unit selection process and the future need for high resolution typing in CBT.

2. The MHC (Major Histocompatibility Complex)

Tissue compatibility is determined by the major histocompatibility complex (MHC), also known as the HLA system

in humans, a cluster of genes located on the short arm of chromosome 6, extending about 3.6 Mb, that play a fundamental role in the acceptance and rejection of transplanted tissues [11]. The MHC is the most gene-dense region of the human genome and encompasses almost 300 genes and pseudogenes, situated in three regions called the class I, class II and class III regions. About 20% of the proteins coded by the MHC have immune-related functions [12]. Immune responses against HLA incompatibility represent a major barrier to hematopoietic stem cell transplantation (HSCT) [13, 14].

The class I region encodes the classical HLA molecules HLA-A, -B and -C, the nonclassical HLA-E, -F, -G, and class I-like molecules MICA and MICB. The class II region comprises the HLA-DR region (containing the DRA, DRB1, and depending on the haplotype DRB3, DRB4, or DRB5 genes), the HLA-DP region (containing the DPA1, DPB1 genes), the HLA-DQ region (containing the DQA1, DQB1 genes), as well as genes encoding proteins involved in antigen presentation. The class III region comprises genes coding for the complement cascade, cytokines, tumor necrosis factor, lymphotoxins, and heat shock proteins [11].

HLA molecules are expressed on the surface of antigen-presenting cells, displaying peptide antigens for recognition by T-cell receptors. T-cell receptors recognize antigens only if presented in the form of peptides bound to self MHC molecules, a concept known as MHC restricted recognition.

Class I molecules are constitutively expressed at varying levels on most nucleated cells and platelets. They consist of a polymorphic transmembrane α-chain (encoded by the corresponding MHC gene) which is associated with and stabilized by a nonpolymorphic $\beta 2$ microglobulin chain, coded by a gene located on chromosome 15. The class-II molecules are restricted to cells of the immune system and consist of two MHC-encoded transmembrane polymorphic glycoproteins, the α and β chain-the latter being the more polymorphic. The structure of HLA Class I and Class II molecules is similar, with most of the polymorphism located in the peptide binding groove. The HLA class I molecule peptide-binding groove can bind peptides that are 8–10 amino acids long whereas HLA class II molecules bind longer peptides (12–24 amino acids). $CD4^+$ T cells recognize antigens presented by class II HLA molecules and $CD8^+$ T cells recognize antigens presented by class I HLA molecules.

The HLA region is the most polymorphic currently known in the human genome. According to the World Health Organization Nomenclature Committee for the HLA System, at the March 2012 update [15] (http://www.ebi.ac.uk/imgt/hla/) there are 1757 HLA-A, 2338 HLA-B, and 1304 HLA-C alleles.

The set of HLA alleles inherited from one parent is referred to as a *haplotype* and is located on one chromosome, for example, the A1-B8-DR3 or the DRB1*15:01-DQB1*06:02 haplotypes. Linkage disequilibrium (LD) a hallmark for MHC, means that certain alleles occur together with a greater frequency than would be expected by chance. This is more frequently observed between closely located loci [11]. Certain haplotypes are common in particular ethnic groups. In hematopoietic cell transplantation from an unrelated donor, the probability of identifying an HLA-matched donor is higher when the patient and donor originate from the same ethnic group [16].

Because of the great polymorphism of HLA molecules, it became clear that serologic typing techniques were completely inadequate to cover all the diversities present in the HLA system. The serology-based method (microlymphocytotoxicity) is still in use for low resolution typing in many laboratories and to clarify the absence of some null alleles [17, 18]. The use of DNA-based techniques for HLA typing moved the field forward. DNA typing methods, are based on the nucleotide sequence information of the polymorphic DNA segments, using PCR technology. A number of HLA typing methods have been developed, mainly using PCR-SSP (sequence specific primers), reverse PCR-SSOP (sequence specific oligonucleotide probes), hybridization on solid support (microbead arrays), or sequence-based typing [18].

Low resolution (LR) referred to as generic typing, or 2-digit typing, corresponds to the identification of broad families of alleles (e.g., A*02) and is the equivalent of serological typing (A2). Medium resolution (MR) tissue typing techniques can define specific allele groups and subtypes. High resolution (HR) or 4-digit typing discriminates the individual alleles in each serotype (e.g., A*02:01) and resolve the tissue type to allele level, with no ambiguity [15]. The use of NMDP (http://bioinformatics.nmdp.org/HLA/hla-res-idx.html) codes can be helpful in this setting. It is recommended that selection of an unrelated donor is based on these first two sets of digits and in a second level of selection to use high resolution typing [19].

3. Related Cord Blood Transplantation

Although the primary interest in CB is an alternative unrelated donor source, CB has been used in related transplants for both malignant and nonmalignant diseases [19–21], performed almost exclusively in children. In an update of the Eurocord experience, with a median followup of 41 months after related CB transplantation for children, the survival estimate was $47 \pm 5\%$ in patients with malignancies ($n = 96$), $82 \pm 7\%$ in patients with BM failure ($n = 33$), 100% in patients with hemoglobinopathies ($n = 52$), and $70 \pm 15\%$ ($n = 10$) in patients with inborn errors of metabolism or primary immunodeficiencies [22]. By matching the Eurocord and International Bone Marrow Registry (IBMTR) [23] results of CB transplantation from HLA identical sibling donors ($n = 113$; median age, 5 years) with the results of BM transplantation (BMT) from HLA identical sibling donors ($n = 2052$; median age, 8 years), it seems that despite the lower incidence of neutrophil recovery at 1 month after CBT compared to BMT (89% versus 98%, resp.), there were no differences in 3-year survival rates (64% versus 66%, resp.), whereas incidence of grade III-IV acute GVHD and probability of chronic GVHD (3 years) were lower after CBT [24].

Related CB transplantation in patients with hemoglobinopathies, offers a probability of success comparable to that offered by BMT and is associated with a lower risk of both treatment-related mortality (TRM) and chronic GVHD, as it

has been reported previously [24]. Based on this, Locatelli et al. [25], recommend collection and freezing of CB units in families in which a child is affected with genetic or hematological disease. In 2003, Reed et al. [26, 27] reported on their successful banking initiative of sibling donor CB for children with hematologic disorders, despite the challenges associated with remote-site collections. The Minesotta Group reported a case of successful CB transplantation for a patient with Fanconi anemia from unaffected HLA genotype-identical sibling selected using preimplantation genetic diagnosis [28]. The practice of preimplantation selection of HLA matched siblings for transplantation has since been established [29].

β-thalassemia is one of the most common single-gene inherited conditions in the world, with a particularly high prevalence in Mediterranean countries, including Greece. The Hellenic Cord Blood Bank, stores CB from healthy siblings of patients with β-thalassemia major. In collaboration with St Sofia Children's Hospital Stem Cell Transplant Unit, eight HLA matched units were released for transplantation and were used alone or in combination with reduced volume bone marrow from the same donor; engraftment was achieved in six out of these cases and all patients survived with 7/8 patients thalassemia-free [30].

4. Unrelated Cord Blood Transplantation in Children

The Cord Blood Transplantation Study (COBLT) [31], has reported the clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. All 193 patients had at least a 3/6 HLA match by low-resolution HLA-A, -B, and high resolution HLA-DRB1. The overall survival at 1 year was 57.3%, and grade III/IV aGVHD and cGVHD incidence was 19.5% and 20.2%, respectively. Higher TNC dose significantly improved engraftment. Retrospective high resolution (HR) HLA typing and the subsequent multivariate analysis revealed that while the level of original HLA match had no impact on the occurrence of grade II–IV or grade III-IV aGVHD, if the pair were matched for fewer than 5/6 alleles (HR) the probability of developing grade III/IV GVHD was significantly higher. Concerning overall survival, although there seemed to be a trend for survival advantage for 6/6 matched patients for both LR and HR typing the size of the cohort does not allow to draw definitive conclusions. The authors suggest selecting CB units that are at least 4 of 6 by LR typing at class I loci and HR typing at HLA-DRB1. Another concern is that even if HR matching decreases GVHD, overall survival may not be affected because of competing contributions of GVHD and graft-versus-Leukemia. Further analysis of larger series will provide more conclusive results regarding the impact of HLA matching on CBT.

On the other hand, for patients with non-malignant diseases the use of unrelated CB from HLA-mismatched unrelated donor will require a larger study, regarding engraftment, survival and GVHD.

In patients with hemoglobinopathies, the risk factors like the donor/recipient mismatching and cell dose, are probably amplified by the effect of multiple transfusion exposures, that might sensitize the recipient to donor alloantigens.

In the case of severe Sickle cell disease (SCD), the cytokine milieu of SCD, which activates the inflammation and the immune activation might also promote a host-versus-graft reaction and interfere with engraftment even after myeloablative preparation [32]. In a recent phase II (BMT CTN) study of the toxicity and efficacy of unrelated donor HSCT in children with SCD, using a reduced-intensity condition regimen, one patient had 6/6 HLA antigen matching with his donor (using low-intermediate resolution typing for HLA-A-B and high resolution for HLA-DRB1), while seven patients had 5/6 HLA antigen mismatching. The median post-thaw infused $CD34^+$ cell dose was 1.5^*10^5/kg. All patients achieved neutrophil recovery in median 22 days. Two patients developed grade II acute GVHD, one of these chronic GVHD and died 14 months postransplantation. According to the data a number of modifications should be done to improve the rate of engraftment after CB transplantation for severe SCD [32].

The use of CB from unrelated donors in β-thalassemia patients resulted in 77% survival in a study of 36 cases [33]. In another study investigating the feasibility of using CBT from unrelated HLA mismatched donor in 5 children with β-thalassemia major, all patients showed grade II or III acute GVHD and none developed extensive chronic GVHD. All patients were alive at a median followup of 303 days after transplantation with complete donor chimerism and transfusion independence [34].

There is a limited experience of CB transplantation in pediatric cases with idiopathic severe aplastic anemia (SAA). Information has mostly been included in registry data with very few details available [35, 36]. In a study from the Children's Hospital in San Antonio, nine children with SAA were transplanted with CB units selected from various CB banks of the USA and the choice was based on the best HLA compatibility, with at least four out of six loci matching. HLA-DRB1 compatibility between the donor and the recipient was in complete priority. At a median followup of 34 months, seven patients are alive and transfusion independent [37]. A simultaneous infusion of $CD34^+$ haploidentical cells seems to improve CBT outcome for patients with SAA [38].

In pediatric patients with severe SCID there is a big discussion about the use either of mismatched related stem cells or unrelated cord blood for transplantation. According to a retrospective study on behalf of Eurocord and the Inborn Errors Working Party of the European Group for Blood and Marrow Transplantation, and although only 4 centers performed both techniques, the results did not differ significantly in terms of 5-years survival despite a higher incidence of chronic GVHD in CBT recipients [39]. CB transplantation has also been shown effective in metabolic diseases [19] in which time from diagnosis to definitive treatment may represent a crucial period to prevent further progression of the disease. The group at Duke University has reported outcomes in 20 children with Hurler syndrome who received condition regimen followed by infusion of unrelated 1, 2, or 3 HLA antigen mismatched CB. With a median

followup of 905 days 17–20 children are alive with complete donor chimerism [40].

In a pilot study of Duke University, conducted in order to determine the safety and feasibility of intravenous administration of autologous umbilical cord blood in young children with acquired neurologic disorders, the results showed that the intravenous infusion of autologous CB is safe and feasible in young children [41].

The comparison of the results of CB and BM transplantation from unrelated donors in children is of paramount importance. It is now accepted that unrelated CB is an efficient alternative to matched unrelated BM in children and the start of a simultaneous search for BM and CB unrelated graft is supported. The final selection of unrelated donor BM versus CB should be based on the urgency of the transplant, the cell dose and HLA matching of the BM and CB unrelated donor. Moreover, CB is advantageous for children requiring urgent transplantation [9].

5. Unrelated Cord Blood Transplantation in Adults

The first unrelated cord blood transplantation was performed in 1996 and since then, more than 20.000 patients have undergone CB transplantation. In the adult setting, in a retrospective analysis of the data concerning 1525 patients with acute leukemia the results revealed that the leukemia-free survival after CBT with 4 to 6 of 6 HLA match was comparable to 7-8/8 allele-matched BMT, with grade II-IV acute and chronic GVHD and chronic GVHD lower in CBT recipients than in PBPC and BM recipients respectively. The issue of further analysis of the impact of HLA matching on transplant outcome was not addressed in this study, as for CB cell dose and not HLA matching is considered to be the limiting factor for its use: the use of a 4–6/6 match CB is considered the equivalent of a 7/8 allele matched unrelated donor when a fully matched donor is unavailable [42].

The feasibility of identifying HLA-matched donors depends on the HLA antigens of the patient and the size of the donor registries [43–45]: every patient has a mismatched donor. Intense efforts have been made to determine the "permissive" of HLA mismatches that do not increase post-transplant risks. Data for the outcomes of 1202 CB transplantations, facilitated by the New York Blood Center National Cord Blood Program, showed important differences in the small subgroups of patients with unidirectional mismatches. The graft-versus-host direction only (GVH-O) and rejection direction only (R-O) mismatches were present in 4.8% and 3.3% of the cases, respectively. According to their results, recipients of transplantation with GVH-O mismatches had neutrophil and platelet engraftment rates that were comparable to those of recipients of transplantations matched in HLA-A, -B, and -DRB1. With GVH-O mismatches, the time to engraftment was significantly faster than transplantation with R-O mismatches. In addition, patients with hematologic malignancies given GVH-O grafts had lower transplantation mortality and treatment failure compared to those with matched CB grafts [46, 47]. The practical implication is that including HLA mismatch direction in search procedures permits easy identification of grafts with unidirectional mismatches, allowing to give priority to GVH-O and to avoid R-O grafts [47].

Since the identification of HLA-C as a classical transplantation antigen [48], donor mismatching for HLA-C has been shown to be a risk factor after myeloablative, nonmyeloablative, unrelated donor, cord blood, marrow, and peripheral blood stem cell transplantation. A retrospective study for the effect of donor-recipient HLA matching at HLA-A, -B, -C, and -DRB1 on outcomes after CBT for leukemia and myelodysplastic syndrome, underlines the importance of HLA-C matching in CB transplantation [49]. Several reports on the association between HLA matching and survival after adult unrelated donor transplantation, showed higher transplant related mortality for transplantation HLA-A, -B, and -DRB1 matched and HLA-C mismatched, or mismatched at a single HLA-A, -B, or -DRB1 locus and mismatched at HLA-C, and transplantations mismatched at a single HLA-A, -B, or -C locus and mismatched at DRB1 [50, 51]. HLA-C is an important model for understanding differential risks conferred by allele and antigen mismatches [52]. Donor recipient pairs mismatched at HLA-C are likely to be mismatched at HLA-B because of the high degree of linkage disequilibrium between these loci [53]. Studies of prognostic factors with larger series of adults given a CB transplant are still missing and any attempt to explain the different outcomes among these series is premature.

There is data analyzing the impact of administering a CB unit that shares a non inherited maternal HLA antigen (NIMA) with a mismatched HLA antigen in the recipient, for patients with hematologic malignancies treated with CB transplantation [54]. These noninherited maternal antigens may define "permissive" HLA mismatches and could be used to extend the genotypes of suitable matches for particular donors or CB units. Rocha et al., demonstrated that CB transplants matched for NIMA were associated with lower transplant related mortality and decreased relapse. A study by the CIBMTR, NMDP and Eurocord [55] found that NIMA matched CB transplantation resulted in superior survival and disease-free survival compared to equivalent NIMA mismatched transplantation. At the present time the role of NIMA matching in the engraftment in CB transplantation is not very clear and requires additional investigation.

From previous studies ABO incompatibility is not considered as a barrier to successful allogeneic HSCT, even though it can be associated with several immunohematologic complications, like delayed red blood cell engraftment, red cell aplasia, or hemolytic anemia. However, red blood cell alloimmunization was recently reported as an independent predictor of HLA alloimmunization [56, 57].

A retrospective analysis of pretransplantation sera from unrelated donor HCT recipients, showed that the presence of donor-directed, HLA specific alloantibodies was significantly associated with graft failure [58]. A recent analysis of sera from 386 myeloablative CB transplant recipients showed that the presence of donor-specific antibodies (DSA) correlated with significantly lower neutrophil recovery compared with those who lacked alloantibodies [59]. The presence of

preformed DSA in double CB transplantation is predictive of higher graft failure rates and high incidence of mortality [60]. Until recently, in vitro crossmatching was used to determine compatibility between donors and recipients, and the relationship between a positive crossmatch and graft rejection in allogeneic transplantation is well established. There is strong evidence, that there is a relationship between the presence of preformed DSA and a positive crossmatch, therefore units that elicit an intense antibody response should be avoided [60, 61].

There is currently little clinical evidence suggesting an important clinical impact for HLA-DR-DQ or DP matching for CB transplantation as well as other non HLA loci like Minor Histocompatibility antigens, Killer immunoglobulin-like receptors (KIR), cytokines, chemokines, and immune response genes.

6. Double CB Transplantation

In order to overcome cell dose limitations, improve engraftment rates, and immune reconstitution, a strategy consisting of administering two partially matched CB grafts called double CBT (dCBT) has been implemented. The University of Minessota program [62], a pioneer of double or sequential CBT using a nonmyeloblative regimen, has published impressive results. Although dCBT (like single CBT) shows delayed engraftment compared to other donor sources, the higher TRM is counterbalanced by lower relapse rate.

Avery et al. [63] examined the effect of HLA match on engraftment after dCBT. In almost all dCBT outcomes, single-unit dominance is observed. No relationship was found between CB/recipient match and unit dominance, even at the allelic (HR) level: a better HLA matched unit at high resolution was not more likely to become the dominant unit. Donor engraftment, is not influenced by the level of match (either at antigen or allelic level) between the two units administered; although high unit-unit match is associated with elevated initial engraftment it has no bearing to eventual graft failure. The authors recommend infusing two units with a cell dose in each unit adequate for engraftment, and 4/6 to 6/6 HLA matching to the recipient at antigen level at class I and allelic level at DRB1.

The influence of HLA matching on engraftment as well as other transplantation outcomes after double-unit CBT, should be readdressed in the future, when a very large number of cases will be available for study. Therefore, although double unit grafts have been widely adopted as a simple strategy to augment graft cells dose in unrelated donor CB transplantation, there is still little information to guide transplant centers in the selection of the graft.

Finally, more recently, it has been observed, that the percentage of viable $CD34^+$ cells after thaw can vary significantly according to the bank of origin, and poor viability units were unlikely to engraft [64]. Querol et al. [65], have similarly reported variable quality between units. This raises the possibility that part of the benefit of dCBT is that, by transplanting two units, we increase the chance that at least one good quality unit, with high engraftment potential, is infused. Given that unit quality is one of the most important considerations in CBT today, the field must determine how unit quality can be reliably measured and ensured, and how poor quality units are to be investigated and/or eliminated.

7. Cord Blood Unit Selection

With the number of cryopreserved CB increasing and the better understanding of the factors influencing transplant outcome (cell dose, HLA match, $CD34^+$ dose, etc.), a need has arisen for better strategies regarding unit selection. Organizations like the NMDP have published guidelines and transplant centers worldwide have established their own set of criteria regarding donor selection, adapted to the transplantation protocols they use and the type of patient they cater to.

NMDP strategy [53] for cord blood unit selection indicates that all patients should receive a cell dose of $>2.5 \times 10^7$ NC/kg. In case of double CBT, each CB should have a cell dose of $>1.5 \times 10^7$ NC/kg. Moreover, the patient should receive a 4/6 or better A, B, DR HLA match. For dCBT, the units should also be 4/6 or better HLA match to each other and if units have an adequate cell dose of $>2.5 \times 10^7$ NC/kg, a 6/6 match is preferable to a 5/6 matched unit. A very important parameter, is to avoid HLA mismatches at loci in which patients have preformed HLA antibodies. It has also been suggested that if maternal typing is available, a CB with a NIMA-shared antigen should be preferred.

HLA matching for unrelated cord blood transplantation generally focuses on three loci (HLA-A,-B, -DRB1). Although selection currently is done to maximize matching at the antigen-level for HLA-A and -B, and at the allele-level for -DRB1, all three loci plus HLA-C are being typed by many centers at high-resolution. In a recent retrospective analysis from NMDP/CIBMTR and Eurocord [49], transplants mismatched at HLA-C were associated with higher transplant-related mortality compared to transplants matched at HLA-C; among transplants mismatched at two loci, mismatching at HLA-C and -DRB1 was associated with the highest risk of mortality. This study suggests that extended HLA matching may yield better outcomes after cord blood transplantation, although HLA match does not predict survival nor the predominant cord [66].

Gluckman and Rocha [9] reported a higher incident of graft-versus- host-disease (GVHD) and longer platelet recovery with both Class I and Class II mismatches. The effect of HLA mismatch is most important when the cell dose is low, and transplant centers are addressing the limitations in cell dose by combining two cord blood units for transplantation. Recent studies [67] examined the relationship between cell dose and HLA match in 1061 patients undergoing cord blood transplantation. Both cell dose and HLA match were independent predictors of transplant-related mortality. Patients receiving 6/6 matched CB unit had improved outcomes, regardless of cell dose. A 4/6 matched CB with cell dose $>5.0 \times 10^7$ NC/kg was comparable to a 5/6 matched CB unit with cell dose $2.0–5.0 \times 10^7$ NC/kg. Although no consensus has yet been reached concerning intra-unit HLA match in dCBT, current practice is to maximize matching of the two units to the recipient at

the antigen-level for HLA-A and -B, and at the allele-level for DRB1 with a minimum of 4/6 match [68].

8. Current Opinion in Cord Blood Banking

Since the first human CB transplant performed in 1988, CB banks (CBB) have been established worldwide for collection and cryopreservation of CB for allogeneic hematopoietic stem cells transplant [69]. CB banking includes the following phases: (1) donor recruitment, consent, and medical evaluation of the donor; (2) CB collection; short-term storage and transportation; (3) processing, testing, cryopreservation, and storage; (4) release of CB unit to transplant center; (5) quality assurance according to FACT/NETCORD standards [27].

The Netcord Foundation (http://www.netcord.org/) is a European nonprofit cooperative network of large experienced CB banks, formally established in 1998 in order to improve the quality of the grafts. The inventory of Netcord currently has more than 300.000 cryopreserved CB units ready to use, with more than 8.624 grafts shipped.

Eurocord was established in 1995 and its principal objectives were to collect data provided by CB banks and transplant centers. Eurocord (from 1988 to October 2010), has collected feedback on 6736 transplanted CB units from transplant centers in Europe and other countries. In the USA, the National Marrow Donor Program (NMDP) has established a similar CB bank network.

International search systems have been established in order to aid transplant centers to locate eligible CB and/or adult unrelated donors (AUD). These include the Bone Marrow Donors Worldwide (BMDW): a database with HLA data and other information pertaining to CB characteristics from registries and CBB worldwide, and the EMDIS (European Marrow Donor Information System) that is a network connecting 26 registries with both CB and AUD. The two systems are complementary and account for approximately 80% of the international transplant activity [70, 71].

From collection and processing through transplantation and followup, a CB quality assurance program establishes a series of controls, quality monitors, and mechanisms that ensure product uniformity, preventing errors, and promoting continuous process and improvements. This approach has elevated the fields of CB banking and transplantation to new issues in regard to quality and process control. The Netcord Foundation in cooperation with FACT (Foundation of Accreditation of Cellular Therapy) has developed standards [72] for CBB that have been adopted by the World Marrow Donor Association (WMDA) and other National and International transplant organizations.

The optimal number of CB units stored in order to provide any patient with a minimum 4/6 HLA matched unit, is not really known, but should approach 9 per 10.000 inhabitants [70]. An issue that should be addressed is the HLA haplotype content of the units stored: it should not only cover the commonest haplotypes of the population covered by the CBB, but also a variety of rare haplotypes or haplotypes characteristic of ethnic minorities. Targeted recruitment directed towards minorities is one of the measures already taken by several large CBB. Another measure, might be the use of HLA as a selection criterion by CB Banks, as volume unit or prereduction nucleated cell number, in order to store units not only with the most common HLA haplotypes but also for rare ones. The target would be to have an overrepresentation of rare haplotypes compared to the more common ones, making it easier to find a reasonable match for everyone, although the practical issues would be difficult to overcome.

9. Conclusions

The experience of last 20 years indicates that CBT is a valid alternative method for BM and PBSC transplants. The main advantage of UCB grafts is the low rate of GVHD in the presence of higher HLA disparity, while delayed engraftment is still a mayor disadvantage due to limited cell dose. The current consensus is that CB should be at least 4/6 HLA matching for HLA-A, -B at the antigen level, and HLA-DRB1 at the allelic level. The role of additional loci as well as the impact of each individual locus remains to be determined by international studies and extended meta-analysis of large numbers of cases.

Considering the additional increasing molecular understanding of most diseases, allogeneic stem cell transplantation is headed towards a next generation of transplantation procedures: the individual adaptation in terms of graft source, engineering, and post-transplant immune interventions depending on the type of disease and underlying genetic alterations of donors and patients. Furthermore, it will allow to combine the beneficial effects of several allogeneic transplantations strategies [73], such as the early haplo-mediated neutrophil recovery, the targeted antileukemia effect of NK cells (KIR mismatch) and T-cells after selected haplo-HSCT and the long term excellent T-cell recovery after CB transplantation, but also to predict, in case of a double CB transplantation, which unit will remain as the long-term graft. All this would provide a crucial advantage for patients in need of grafts with unique genetic features such as mutations in the CCR5-coreceptor rendering carriers resistant to certain types of HIV infection: taking advantage of such types of grafts would allow curing patients with hematological malignancies and co-infection with HIV [74, 75]. Cord Blood, with its immediate availability and the possibility of having genotypically well characterized units, is a prime candidate for these applications and in the future other biological markers influencing transplant outcome or providing an advantage to carriers could be added to the selection criteria used.

Much research is ongoing to investigate the potential use of UCB stem cells in regenerative medicine. Clonal lines of multipotent cells (called the multilineage progenitor cell, MLPC) have been established from full term UCB, which can expand and differentiate into cells representing all three germinal layers. Recently, it has been shown that human unrestricted somatic stem cells (USSC's) from umbilical cord blood represent pluripotent, neonatal, nonhematopoietic stem cells with the potential to differentiate into osteoblasts, chondroblasts, adipocytes, hematopoietic, and neural cells. The mesenchymal stem cells (MSC) derived from UCB or

umbilical cord (Wharton's Jelly) with their differenciation potential and immune-modulatory properties are of interest in the field of cellular therapies and regenerative medecine. MSC mediated immunosuppression, after simultaneously MSC transfusion and HSCT, has been shown to contribute to faster engraftment [76] and can be used as anti-GVHD prophylaxis [77]. CB is also a convenient source of induced pluripotent stem cells [78].

As the potential uses of cord blood extend beyond HSCT, the notion of CB banking will have to be reinvented. Cellular therapies and regenerative medicine have different immunological considerations and HLA will have a role to play that will be different: that of providing individuals with well-suited therapies. In the years to come, the better understanding of the biology of CB derived stem cells, in conjunction with new technologies will provide additional tools for the realisation of both exciting new research and novel therapeutic applications.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 960280, 5 pages
doi:10.1155/2012/960280

Review Article

Th17 Mediated Alloreactivity Is Facilitated by the Pre-Transplant Microbial Burden of the Recipient

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Received 27 July 2012; Accepted 18 September 2012

Academic Editor: Bronwen Shaw

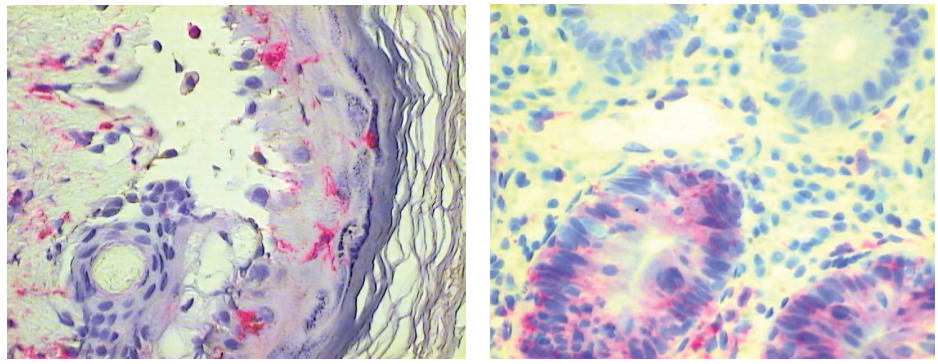
Acute graft-versus-host disease (aGvHD) is a major complication after hematopoietic stem cell transplantation (HSCT) and severity of aGvHD is associated with biological and genetic factors related to donors and recipients. Studies on inflammatory pathways involved in aGvHD have shown a significant impact of the gut microflora on aGvHD development giving increasing evidence in the understanding of the response of innate and adaptive immunity to microbial products. Cytokine deregulation may increase or reduce the risk of aGvHD. Damage of tissues affected by aGvHD reflects the immunological cascade of events in this disease.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a clinically accepted procedure in some hematological malignances, aplastic anemia, and inborn errors. It is rather a complex procedure, associated with both the adverse effect aGvHD and with the presence of beneficial alloreactivity, as it is graft versus leukemia or versus cells with inborn error reaction [1–4]. Alloreactivity influences both hematological and immunological recovery. Both alloreactivity and recovery of blood cells take place in an environment full of microbial agents in a latent form or colonizing/invading the host. Innate and adaptive immunity competence prior to and after HSCT secure an event-free course after HSCT with respect to that.

1.1. Biology of Acute GvHD. Damage of the gastrointestinal tract during the acute phase of GvHD plays a major pathophysiological role in the amplification of this systemic disease. Several experimental and clinical observations highlight the role of effector cells of the immune system migration into the skin and gastrointestinal tract in the pathobiology of aGvHD [5]. Mice are the most often used animal model of GvHD. Differences in age, sex, genetic matching, and also gut microbiota of the mice are found to be the main players in pathophysiology of GvHD [6].

One of the first reports describing the microbial environment of the recipient as an important cofactor of gut aGvHD development was presented by Van Bekkum et al. [7, 8]. In their studies they compared the fate of conventionally and germ-free housed mice after whole-body irradiation and MHC incompatible bone marrow cell transplantation. Enteric aGvHD was less frequent in germ-free mice and in mice receiving antibiotic prophylaxis as compared to conventionally transplanted animals. The authors concluded that antigenic epitopes of microorganisms shared with gut epithelial cells may promote alloreactivity. These observations indicated that lymphocytes sensitized against microbial antigens may cross-react with epithelial cells in the gut, promoting aGvHD. Experimental studies demonstrated that loss of integrity of the gastrointestinal tract plays a major role in experimental GvHD [9]. Intestinal microflora, their



(a) (b)

Figure 1: (a) HLA-DR expression on antigen presenting cells in the epidermis of the skin (+60 days after HSCT) and (b) HLA-DR expression on colon epithelial cells (+33 days after HSCT) affected by aGvHD (red staining with Permanent Red, magnifications 400x).

antigenic challenge, and released endotoxins constitute part of the microenvironment and can serve as potent triggers of inflammation in GvHD [9].

1.2. Innate Immunity and aGvHD. The studies on the role of gut microflora in initiation of aGvHD help in understanding the role of the innate and adaptive immune response evoked by microbial products in this disease [10]. Conditioning regimen damage of the gut and concomitant release of endotoxins and lipopolysaccharides (LPS) from colonizing the gut microbes activate innate immunity via Toll-like receptors (TLRs), which starts a cascade of events leading to cytokine storm, which constitutes part of the aGvHD pathomechanism [9, 11, 12]. Ligation of intestinal TLR9 by bacterial DNA increases the risk of aGvHD. TLR9 knockout mice have aGvHD of a reduced activity and intestinal damage [11]. The impact of bacterial sensing via TLRs in gut aGvHD was analyzed in an intestinal mice model which shows that MyD88 (myeloid differentiation primary-response protein 88)-dependent TLR9 signaling of bacterial DNA is essential for induction of apoptosis and cell infiltrations in the gut during aGvHD [13]. Indeed, the use of oligonucleotide (iODN) 2088, which inhibits TLR9 activation in vitro, ameliorates the symptoms of gut aGvHD in mice [13]. In contrast, mutations in TLR4 (which encodes LPS receptor) have been shown to be a biological factor reducing the risk of GvHD in experimental studies [14].

Manipulation with gut microflora in favor of *Lactobacillus rhamnosus* GG [15] makes aGvHD less aggressive. Very recent experimental and clinical studies demonstrated that microbial chaos early after HSCT and loss of intestinal flora diversity are a potential risk factors for subsequent aGvHD development [16]. In clinical practice intestinal bacterial decontamination with metronidazole and ciprofloxacin significantly reduces the severity of gut aGvHD [17], which supports the notion that intestinal microflora play a role in the pathogenesis of aGvHD.

The NOD2/CARD15 protein, restricted to intestinal epithelial cells and monocyte/macrophage lineage [18], plays a role in the innate immune response to bacterial infections in the gastrointestinal tract. It is at present known that NOD2/CARD15 gene mutations found in patients undergoing HSCT make them more susceptible to aGvHD [19]. The cumulative incidence of 1-year transplant-related mortality and the prevalence of severe gut aGvHD affected 49% of patients with NOD2/CARD15 gene mutation as compared to 20% incidence in those without NOD2/CARD15 gene mutation. If the mutation affects donors this proportion increases to 59% and to 83% if both recipient and donor have the gene mutated [20]. Our observations also show that NOD2/CARD15 gene mutation is associated with susceptibility to severe GvHD grade III-IV [21]. Moreover, we found that mutations in the NOD2/CARD15 gene influences the level of Th17 in blood in such a way that patients with NOD2/CARD15 mutations had lower blood values of Th17 at the time of hematological recovery in the aGvHD group [22].

2. Pathophysiology of aGvHD

The conditioning regimen causes tissue damage and as a consequence several proinflammatory cytokines including IL-1 and TNF-α, and a set of chemokines, CCL2-5 and CXCL9-11, are released, thereby increasing expression of adhesion molecules, MHC antigens and costimulatory molecules on the host antigen presenting cells (APC) [1, 23]. Host APC, which survive the conditioning regimen damage, become activated and capable of confronting the transplant material antigens (Figure 1). Activation of donor T cells after interaction with host APC leads to their proliferation, differentiation, and migration. In the subsequent effector phase mononuclear cells invade the target tissue and accumulation of these cells leads to tissue destruction (Figure 2) [23, 24].

It is known, also from our own experience, that anti-CD52 monoclonal antibody (MoAb) (Campath-1H), if used as part of the conditioning regimen, greatly decreases the risk of aGvHD (unpublished). Anti-CD52 MoAb has a unique property to destroy not only lymphocytes but also APC [25].

In afferent phase 1 of aGvHD LPS of Gram-negative bacteria are the main stimulators of proinflammatory cytokines

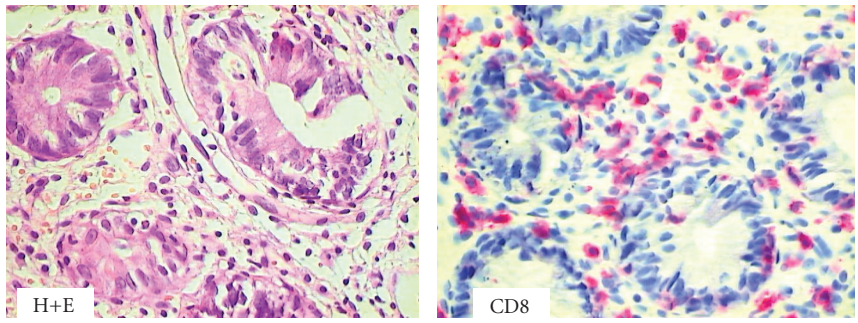


Figure 2: Colon biopsy specimen harvested at 33 days after HSCT from patient with clinical symptoms of aGvHD. Hematoxylin and eosin (H+E) staining documented destruction of colon crypts, and immunocytochemistry illustrate CD8+ cells invading damaged crypt epithelium (H+E magnification 200x, red staining with Permanent Red, magnifications 400x).

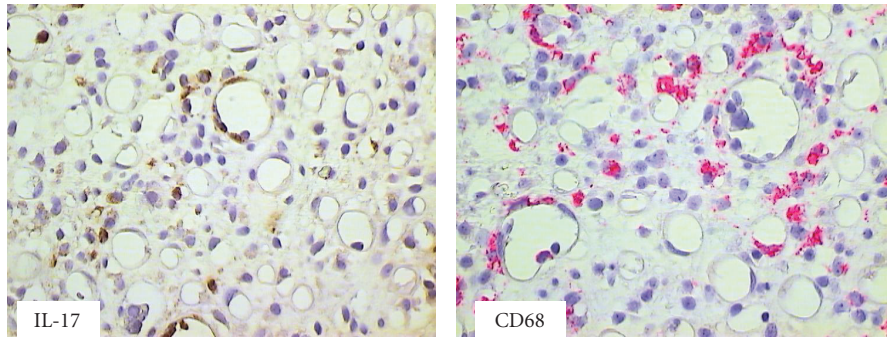


Figure 3: Colon biopsy specimen harvested at day 63 after HSCT from patient with clinical symptoms of aGvHD. IL-17 producing cells and macrophages CD68+ were seen within cellular infiltrates (brown staining with diaminobenzidine-tetrahydrochloride (DAB) and red staining with Permanent Red, magnifications 400x).

and chemokine receptors. The intensive conditioning regimen induces apoptosis and consequently epithelial cell damage, allowing LPS to enter the systemic circulation, activating host APC, which facilitates alloreactivity, leading to aGvHD [9, 26].

Activated T cells proliferate and secrete cytokines [1]. Th1 cells contribute to the cytokine storm associated with aGvHD, while Th2 cytokines may mitigate the impetus of alloreactivity [6]. Indeed, in our early studies we confirmed the presence of IFNγ, IP-10 as well as TNF-α and IL-6 transcripts in skin affected by aGvHD [27]. IL-2 and IFNγ prime mononuclear phagocytes to produce IL-1 and TNF-α. TNF-α, a powerful inducer of APC in the first phase, activates T cells also in the second phase of aGvHD. Again, the microbial impact plays a role in establishing a vicious circle of infection (TNFα, IL-6) and thus aggravation of aGvHD.

The role of microbial infection in aggravating aGvHD has a long history [28]. Recently we added some more information as to the role of Th17+ lymphocytes, whose differentiation is strongly supported by microbial invasion. It is a step-by-step process starting with TNF-α and IL-1 secretion in phase 1 in response to the conditioning regimen and microbial background. Among proinflammatory cytokines, IL-6 plays an important role in aggravation of aGvHD, especially when the gut is targeted [27, 29]. This cytokine is released and generated during inflammatory processes associated with (i) the conditioning regimen, (ii) the alloreactivity associated inflammation, and (iii) bacterial and fungal infections [27]. C-reactive protein (CRP) is a reading protein of IL-6 and usually reflects microbial invasion. Notably, increase of the serum CRP level may herald gut manifestation of aGvHD [27]. Increase in serum level of IL-6 is seen early after transplant as a result of a cytokine storm described in allogeneic HSCT patients at the period of neutropenia, and then an increase may be again seen during prolonged leucopenia and at that time is usually associated with infectious complications [29]. All these events are responsible for elevation of serum CRP level during the period after HSCT [29, 30].

Following more recent observations it is known that the differentiation process of CD4+ cells into subsets depends on the cytokine milieu in their environment [31]. IL-6, if present, facilitates differentiation of CD4+ cells into Th17 cells [32, 33]. IL-17 is a cytokine of the strongest proinflammatory potential. It is known that differentiation of lymphocytes into Th17 cells may take place in the gut, where microbial products provide strong stimulation for local IL-6 production [34]. Therefore, it is not surprising that in intestinal aGvHD IL-17 producing cells are present among those infiltrating affected tissue (Figure 3) [35]. Local IL-17 production in the gut during aGvHD is seen in patients with extensive diarrhea resulting from profound damage of intestinal epithelium.

Th17 differentiation is guided by IL-6, which constitutes a primary response to bacterial and fungal infections. Th17 cells have as a hallmark receptor CCR6, which in response to their ligand CCL20 (also known as macrophage inflammatory protein-3α, MIP-3α), produced by activated macrophages in the inflammatory area of the gut, facilitates colonization of gut epithelium by IL-17 producing cells, causing severe inflammation [36, 37]. A correlation between the number of Th17 cells and the clinical course of aGvHD supports the notion that Th17 cells are involved in the active phases of aGvHD [38]. Our studies showed that IL-17 producing CD4+ lymphocytes are at a higher proportion in blood prior to aGvHD manifestation and then decrease at the time of full blown aGvHD [39]. These cells are likely marginalized in the affected tissue, exerting their strong pro-inflammatory activity.

In conclusion, the data collected since the pioneering work of Van Bekkum strongly suggest that microbial products influence the risk of aGvHD in all phases of pathobiology of this complication via activation of APC then inducing the local production of IL-6 exemplified by CRP serum level elevation to the effector phase exerted by lymphocytes of Th17 cell characteristics. Pre- and peritransplantation colonization of recipients with bacterial and fungal germs promotes alloreactivity; therefore, microbial surveillance plays an important role in securing an event-free post-transplant course. Bacterial and fungal colonization after transplant involves both Gram-positive and Gram-negative bacteria. However, up to date is not sufficiently defined which microbial populations may exert or protect aGvHD-associated damage and inflammation because both Gram-positive and Gram-negative bacteria may overgrow the intestinal flora and may worse aGvHD [16, 28]. Therefore, both Gram-positive and Gram-negative bacteria can play a role in activation of both innate and adaptive immunity with production of IL-6 with following consequences of the presence of this cytokine which may facilitate pathomechanism of aGvHD.

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 680841, 7 pages
doi:10.1155/2012/680841

Review Article

Role of HLA in Hematopoietic Stem Cell Transplantation

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Received 27 July 2012; Accepted 3 September 2012

Academic Editor: Andrzej Lange

The selection of hematopoietic stem cell transplantation (HSCT) donors includes a rigorous assessment of the availability and human leukocyte antigen (HLA) match status of donors. HLA plays a critical role in HSCT, but its involvement in HSCT is constantly in flux because of changing technologies and variations in clinical transplantation results. The increased availability of HSCT through the use of HLA-mismatched related and unrelated donors is feasible with a more complete understanding of permissible HLA mismatches and the role of killer-cell immunoglobulin-like receptor (KIR) genes in HSCT. The influence of nongenetic factors on the tolerability of HLA mismatching has recently become evident, demonstrating a need for the integration of both genetic and nongenetic variables in donor selection.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has been established as a mode of curative therapy for hematologic malignancies and other hematologic or immune disorders. Hematopoietic stem cell donor selection has been almost exclusively based on selecting an human leukocyte antigen (HLA) identical donor or near-identical donor; however, not all patients are able to find a suitable donor. Advances in HLA testing and matching and understanding donor selection factors are therefore important to improve outcomes of unrelated donor (UD) HSCT. HLAs can elicit an immune response either by presentation of variable peptides or by recognition of polymorphic fragments of foreign HLA molecules. HLA disparity has been associated with graft failure, delayed immune reconstitution, graft-versus-host disease (GVHD), and mortality. Since many patients lack HLA-matched donors, current research is focused on the identifying permissible HLA mismatches. Recently, extensive research has accumulated evidence on the role of each HLA locus mismatch on clinical outcome for UD HSCT, making it easy to search for and select a partially matched donor [1, 2].

In this paper, we will focus on the current understanding of HLA typing and its clinical implications on UD HSCT.

2. HLA Typing

HLA class I and II loci are the most polymorphic genes in the human genome, with a highly clustered and patchwork pattern of sequence motifs [3]. Each individual carries 10 to 12 genes that encode the HLA-A, -B, -C, -DR, -DQ, and -DP. Most of these genes are highly polymorphic, ranging from 13 (HLA-DRB4) to 699 (HLA-B) alleles per locus [4]. Extensive allelic diversity has made, and continues to make, high-resolution HLA-DNA typing very challenging. Over the past three decades, the remarkable extent of allelic diversity at these loci has been shown by molecular genetic analyses, made possible by the development of recombinant DNA technology, chain-termination Sanger sequencing, and PCR amplification [3].

Initially, HLA-DNA typing involved restriction fragment length polymorphism (RFLP) analysis, but this approach had many limitations in terms of workflow and resolution and represented at best a complement to, rather than a replacement for, serological typing [5]. The development of PCR in 1985 allowed for the amplification of the polymorphic exons of the HLA class I and II genes and for the analysis of polymorphic sequence motifs with sequence-specific oligonucleotide (SSO) hybridization probes. Currently available

methods to identify specific polymorphisms or nucleotide motifs include SSO probe hybridization, sequence-specific primer (SSP) amplification, sequencing-based typing (SBT), and reference-strand-based conformation analysis [3, 6]. Both PCR-SSP and PCR-SSO rely on the use of oligonucleotide primers or probes to react and/or detect specific and previously known polymorphic sequence motifs present within the amplified HLA-allele fragment. A major disadvantage is that such methods rely on the screening of a limited number of previously known polymorphisms. Therefore, when a novel allele is present a sample, mistyping can occur, depending on whether the allele possesses a different polymorphism or different arrangement of known polymorphisms. However, SBT uses generic oligonucleotide primers directed towards conserved regions of a locus to amplify the polymorphic exons of all alleles. Although SBT is able to detect previously unknown HLA alleles, it is not entirely capable of resolving novel arrangements of known polymorphisms, a limitation known as ambiguity. This problem can be overcome by separating the alleles by groups or allele-specific PCR, cloning, or by the use of conformational techniques. Conformational methods, such as the Reference-Strand-mediated Conformational Analysis (RSCA), have shown to achieve high-resolution results without the ambiguities seen in the previously mentioned methods [7].

HLA-typing methods convey certain advantages and present various limitations. Matching by high-resolution HLA typing, a more recent and sophisticated method, certainly reduces the risk of immune complications, namely, graft rejection and GVHD along with increased chance of finding a suitable donor [2]. As such, the choice of method is dependent on the intended application and on establishing an appropriate balance of what level of resolution is needed with regards to speed of typing, cost, and human intervention [8].

3. Effect of HLA on Clinical Outcomes after HSCT

3.1. Number of HLA Mismatches. Advances in HLA-typing techniques allowing better matching of donor-to-recipient have improved the prognosis of HSCT. A recent prospective study investigating outcomes after transplant with 10/10 allelic-matched unrelated donors (MUDs) and HLA-identical sibling grafts for patients with standard-risk hematological malignancies showed that overall survival, disease-free survival, transplantation-related mortality (TRM), relapse, and acute GVHD were not dependent on donor type [9]. The similar outcome values for different donor types suggest that well-selected UDs can perform as well as HLA-identical sibling donors. Immune genetic disparity in the donor-recipient pair is associated with a worse patient outcome, mainly due to the high incidence of transplantation-related complications. A direct assessment of the number of HLA mismatches between the donor and the recipient has highlighted its great importance in UD HSCT. As the number of class I and II HLA mismatches increases, the risks of graft failure, GVHD, and mortality increase [10–12]. Indeed, a recent analysis by the Center for International Blood and Marrow Transplant Research (CIBMTR) on patients with hematological malignancies, mainly transplanted with bone marrow cells, has shown that, as compared to patients transplanted from a donor matched at the allelic level for HLA-A, -B, -C, and -DRB1, patients given an allograft from a donor with a single antigenic or allelic disparity had an increased risk of both acute GVHD and TRM [2]. Disparities at two or more loci compounded this risk.

3.2. Permissible Mismatches. The need to broaden the availability of UD HSCT for patients who lack a matched donor has provided a rationale to define permissible HLA mismatches. The most important HLA loci influencing post-transplant outcome of patients given HSCT from UDs are HLA-A, -B, -C and -DRB1 [13, 14]. There have been several large-scale analyses on the role of each HLA locus in non-T-cell-depleted UD HSCT (Table 1). The Japan Marrow Donor Program (JMDP) showed the effect of matching HLA class I alleles on the development of severe acute GVHD and the importance of HLA-A and -B allele matching for better survival [10, 15]. The Fred Hutchinson Cancer Research Center (FHCRC) and the US National Marrow Donor Program (NMDP) reported the importance of HLA class II matching to prevent GVHD and to increase survival [13, 16]. An analysis of NMDP in 2004 indicated that HLA-A allele level mismatching, HLA-B serological mismatching, and DRB1 mismatching are significant risk factors for severe acute GVHD and that disparity in HLA class I and/or HLA-DRB1 increases the incidence of mortality [14]. An analysis of NMDP published in 2007 showed that the impact of HLA-A or -DRB1 mismatch on overall survival was more marked than a mismatch at HLA-B or -C [2]. And recent analysis of Korean data showed the importance of HLA-B and -C locus matching for better survival [11]. However, the above-mentioned reports, as well as others, have produced considerable conflicting results on the causal role of HLA mismatch locus on clinical outcomes.

The significance of HLA mismatching may be related to population-based locus- and allele-specific differences that distinguish ethnically diverse transplant donors and recipients. The International Histocompatibility Working Group (IHWG) studied the impact of individual locus mismatches in different populations [17]. The authors found that a single HLA-A mismatch was poorly tolerated in JMDP transplant recipients, but less detrimental in the non-JMDP population. Conversely, mismatches at HLA-C were well tolerated among the JMDP patients, but poorly tolerated among non-JMDP patients. One explanation for this may be differences in the actual allele mismatches in these separate populations. Morishima et al. [18] reported that the most frequent mismatch found in Japanese patients was HLA-A*0201 and HLA-A*0206 and that this mismatch was deleterious. By contrast, the most common HLA-A*02 mismatch in Caucasians was found to be HLA-A*0201 and HLA-A*0205, and an adverse relationship between this mismatch and transplantation outcomes was not found. The identification of a nonpermissive HLA-allele mismatch combination

Table 1: Effect of HLA mismatching on survival.

Study	Mismatched HLA locus			
	A	B	C	DRB1
Petersdorf et al. [13]	Merged A, B, and C Decreased			Decreased
Morishima et al. [10]	Decreased	Decreased	None	None
Flomenberg et al. [14]	Decreased	Decreased	Decreased	Decreased
Lee et al. [2]	Decreased	None	Decreased	Decreased
Park et al. [11]	None	Decreased	Decreased	None

indicates that the ethnic diversity of the recipient and donor can translate into molecular differences based on HLA alleles, indicating that it is essential to reconcile differences in HLA risk observed among ethnically diverse transplant groups. Analysis of HLA-DPB1 mismatches in this way has lead to interesting findings [19, 20]. Crocchiolo et al. [21] reported a significantly higher 2-year survival in transplants with permissive as compared to nonpermissive HLA-DPB1 mismatches (54.8% versus 39.1%, $P = 0.005$). Similarly, Zino et al. [20] found a significantly higher risk of mortality in patients with nonpermissive DPB1 mismatches compared to those without such mismatches.

3.3. HLA-DQ and HLA-DP. The importance of HLA-A, -B, -C, and -DR in HSCT has been well described, whereas there have been conflicting results as to the clinical significance of HLA-DP and -DQ. Less than 20% of transplants matched for HLA-A, -B, -C, -DRB1, and -DQB1 are also compatible for HLA-DPB1, due to the very weak linkage disequilibrium existing between the DR/DQ loci and the DP locus. Therefore, over 80% of unrelated transplants are performed across the HLA-DPB1 barrier [2, 22]. Furthermore, the low frequency of fortuitous HLA-DP matching hinders a precise analysis of the true independent effects of HLA-DP mismatching except in cases of very large numbers of transplants. Early investigations were conflicting as to the significance of HLA-DP as a classical transplantation determinant. In a recent analysis of 627 HLA-identical sibling transplants, of which 30 were HLA-DP-mismatched due to recombination, HLA-DP mismatch was an independent risk factor for GVHD [23]. Furthermore, Schaffer et al. [24] reported that mismatching for HLA-DP was a risk factor for increased mortality compared to DP matching. Most studies now agree that HLA-DQB1 does not need to be considered in a well-matched donor [10], but evidence supports that there may be an additive effect of a DQB1 mismatch if a mismatch at another locus is present [25]. Taken together, roles of the HLA-DQ and DP loci remain not fully elucidated. However, previous results suggest that when patients have a choice of equivalently matched donors, selection of an HLA-DQB1-matched donor over a mismatched donor may decrease posttransplant complications.

3.4. Level of HLA Disparity. The level of HLA disparity (antigenic or allele level) affects HSCT outcome differently [26–28]. Sequence analyses show that antigenic disparity is frequently associated with more than ten amino acid substitutions in HLA molecules, which can be easily recognized by immunocompetent cells, thereby stimulating an immune response [26]. Allele level disparity most frequently concerns only one or a few amino acid substitutions, which should produce weaker immune stimulations. A linear increase in the number of amino acid substitutions in the disparate HLA molecule may cause significant deleterious effects or be irrelevant in HSCT [26, 29]. However, there are conflicting data concerning the value of selecting an allelic mismatch over an antigenic mismatch. According to Lee et al. [2], there were no significant differences in survival depending on whether the mismatch was allelic or antigenic, except at HLA-C, in which an antigenic mismatch increased transplant risks while an allelic mismatch did not. Similarly, a single-center study from Seattle could not find any apparent difference between allele and antigen mismatches with respect to the number of deaths from transplants, suggesting that donors with a single HLA allele of antigen mismatch may be used for HSCT when a fully MUD is not available for patients with severe diseases not permitting time for a lengthy search [25]. However, the NMDP study found that antigenic mismatch was associated with higher mortality compared to allelic mismatch [14]. They indicated that selection of donors with high-resolution mismatches over those with low-resolution mismatches may lower the rate of posttransplant complications. The analysis of large transplant populations with a diversity of mismatches is needed to further define potential differences between allele and antigen mismatches in post-HSCT complications.

3.5. Tolerable Mismatches. Although HLA-identical donors are now known to be the gold standard, using a donor with a single-allele mismatch has been associated with an equally favorable outcome in certain situations. According to the report of Teshima et al. [30], reduced intensity conditioning (RIC) transplantation from a two- to three-loci-mismatched donor resulted in poor outcome, as shown in conventional HSCT. However, the 2-year overall survival after one-locus-mismatched RIC transplantation was comparable with that of HLA-matched RIC transplantation in high-risk malignancies. In a study of T-cell-depleted RIC transplants, there was no significant difference in overall survival between matched or one-antigen-mismatched grafts [31]. A recent report from the United Kingdom, in recipients of T-cell-depleted RIC transplantation protocols using Alemtuzumab, showed that

transplant outcomes were similar between HLA-matched and, mismatched pairs [32]. As listed above, in settings of T-cell depletion and/or RIC transplantation, the impact of HLA matching may differ and these conditions require further investigation.

3.6. Importance of Disease Stage. It is important to note that the effect of a single-allele mismatch may vary with the underlying diagnosis. In a recent publication on 948 donor-recipient pairs at the FHCRC, it was found that a single-allele mismatch conferred a higher risk of death, but only for low-risk patients, defined as those with chronic myeloid leukemia (CML) within 2 years of diagnosis [25]. By contrast, a single-allele mismatch had no effect on survival among higher-risk patients, such as those with more advanced CML, acute leukemia, or myelodysplastic syndrome. Similar outcomes are reported in a recent report from an Italian group [33]. When only a single HLA mismatch (9/10 matched pairs) was present, the mortality risk was higher than among 10/10 matched pairs in patients transplanted with acute leukemia in the first CR (early stage disease), but not in patients with advanced diseases. These results suggest that the potential benefit of HLA matching was offset by the negative impact of advanced disease. Therefore, if a donor search is highly unlikely to yield matched donors in the early phases of disease, the increased mortality associated with a longer time interval from diagnosis to transplantation must be weighed carefully against the increased mortality associated with earlier HSCT with a mismatched donor, as well as against the chance of disease progression during the prolonged donor search.

4. The Role of Anti-HLA Antibodies

Donor-specific anti-HLA antibodies (DSHAs) have been implicated in graft rejection in solid-organ transplantation, but their role in allogeneic HSCT remains under investigation [34–36]. Controversy exists as to whether DSHAs actually mediate graft rejection or if they are surrogate markers for cellular immunity that cause graft failure [37]. DSHAs cause graft failures in animal models of allogenic HSCT, mainly because the cognate HLA antigens are expressed on hematopoietic stem cells and hematopoietic precursors [38].

The complement-dependent microlymphocytotoxicity assay (CDC) has been the standard method for the detection of anti-HLA antibodies for the last 30 years [39]. The recent introduction of solid-phase assays enabled a reassessment of the role of both HLA class I and II antibodies in organ rejection. Spellman et al. [40] tested archived pretransplantation sera from graft failure patients and a matched-control cohort to evaluate the role of DSHAs in UD HSCT. The presence of DSHAs was significantly associated with graft failure (odds ratio = 22.84; 95% CI, 3.57–infinity), indicating that the presence of pretransplantation DSHAs in recipients of UD HSCT should be considered in donor selection. Similarly, Ciurea et al. [41] found that DSHAs were associated with a high rate of graft rejection in patients undergoing haploidentical HSCT. On the basis of the previously mentioned findings, DSHA identification should be performed in HSCT settings where HLA matching is not complete [42]. Immunoadsorption and plasmapheresis could be considered to desensitize the recipient when no alternative donor is available.

5. Killer Immunoglobulin-Like Receptor (KIR) Ligand Incompatibility

Natural killer (NK) cells and subpopulations of T cells express NK cell receptors. The activity of NK cells is controlled by the recognition of HLA class I molecules on the target cells by NK cell inhibitory and activating receptors [43, 44]. Depending on the type of KIR, ligation by HLA can stimulate or inhibit the ability of NK cells to kill foreign cells, including tumor cells [45]. The coexistence of the incompatibility of both types on the same HLA molecules makes it difficult to show the advantages of KIR-ligand mismatches clearly. The strong immune reactions provoked by T-cell recognition elements on incompatible HLA molecule can probably override the favorable effect of the simultaneous KIR-ligand mismatch [46]. In fact, Farag et al. [47] investigated the effect of KIR-ligand mismatching on the outcome of UD HSCT in the T-replete setting. In that study, patients who received grafts from donors mismatched at the KIR ligand and at HLA-B and/or C but matched at the KIR ligand had similar rates of TRM, treatment failure, and overall mortality. By contrast, Giebel et al. [48] investigated UD HSCT in 130 patients with acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), or CML, who received unmanipulated grafts. The results of that study showed that transplant from KIR-incompatible donors resulted in enhanced overall survival, decreased disease relapse, and increased probability of disease-free survival. When myeloid leukemia patients were selected for analysis, these effects became more prominent, suggesting that patients with myeloid malignancies were more responsive to treatment. More recently, Cooley et al. [49, 50] analyzed the outcomes of 1,409 patients, taking into account the role of KIR-gene variability. Donor KIR genotype influenced transplantation outcomes for patients with AML but not for those with ALL. Compared to donors without KIR mismatches, donors having KIR mismatches showed reduced incidences of relapse and improved disease-free survival. Furthermore, KIR-ligand incompatibility in the graft-versus-host direction in haplotype-mismatched transplants suggests a possible clinical benefit as it may allow early recovery of donor alloreactive NK cells with enhanced antileukemia activity in AML [51].

If KIR mismatch results in graft versus tumor (GVT) effects, one may assume that several mismatches would result in further enhances in the GVT effect. Previous transplant studies commented upon the impact of numerous mismatches compared to one mismatch. Clausen et al. [52] demonstrated that relapse risk was decreased in patients who underwent HLA-identical sibling HSCT who both received high NK cell dose and lacked at least one HLA-B or HLA-C ligand to a present donor's inhibitory KIR. In that study,

transplants with more than two different activating donor KIRs were associated with an increased risk for nonrelapse morality. Similarly, Willemze et al. [53] reported that a higher number of HLA disparities resulted in a decreased incidence of relapse in patients who received umbilical cord blood transplantation.

Collectively, it is clear that the exploitation of NK cell alloreactivity as a therapeutic advantage in HSCT is promising, and certain patients with myeloid malignancies have benefited from allogeneic HSCT. KIR genotyping of several best HLA-matched potential UDs may change clinical practice in the future [54].

6. Conclusion

A donor's HLA match status should be considered to help the physician and patient in transplantation-related risk assessment and in planning treatment options based on those risks. The benefits of high-resolution HLA class I and II typing have been well demonstrated, particularly for posttransplant survival. The current gold standard is a donor matched for 8/8 alleles; however, it is clear that mismatches may be tolerated with regards to survival in some transplant settings and that evidence for permissive mismatches exists. Permissiveness depends not only on the potential adverse effects of HLA mismatches, but also on the urgency of the HSCT, the desirable GVT effect, and the potential efficacy of the alternative therapy available for the patient. Further knowledge on DSHAs, NK cell alloreactivity, and KIR receptors will aid HSCT in becoming safer and more efficacious.

Conflict of Interests

The authors declare they have no conflict of interests.

Acknowledgment

This study was supported by a Grant from the National R&D Program for Cancer Control, Ministry for Health and Welfare, Republic of Korea (0520290-3).

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Hindawi Publishing Corporation
Bone Marrow Research
Volume 2012, Article ID 834040, 5 pages
doi:10.1155/2012/834040

Review Article

What Is the Most Appropriate Source for Hematopoietic Stem Cell Transplantation? Peripheral Stem Cell/Bone Marrow/Cord Blood

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Received 16 June 2012; Accepted 30 August 2012

Academic Editor: Andrzej Lange

The introduction of peripheral stem cell (PSC) and cord blood (CB) as an alternative to bone marrow (BM) recently has caused important changes on hematopoietic stem cell transplantation (HSCT) practice. According to the CIBMTR data, there has been a significant decrease in the use of bone marrow and increase in the use of PSC and CB as the stem cell source for HSCT performed during 1997–2006 period for patients under the age of 20. On the other hand, the stem cell source in 70% of the HSCT procedures performed for patients over the age of 20 was PSC and the second most preferred stem cell source was bone marrow. CB usage is very limited for the adult population. Primary disease, stage, age, time and urgency of transplantation, HLA match between the patient and the donor, stem cell quantity, and the experience of the transplantation center are some of the associated factors for the selection of the appropriate stem cell source. Unfortunately, there is no prospective randomized study aimed to facilitate the selection of the correct source between CB, PSC, and BM. In this paper, we would like to emphasize the data on stem cell selection in light of the current knowledge for patient populations according to their age and primary disease.

1. Trials Comparing Bone Marrow and Peripheral Stem Cell

One of the main reasons for preferring PSC worldwide is the important advantages provided by this method to the donor. These advantages are avoidance of anesthesia, lack of the need for hospitalization or blood transfusion, and very low serious adverse event risk. The largest trial to date comparing these different stem cell sources in HLA matched sibling donor setting was the meta-analysis of IBMTR/EBMT including 536 and 288 patients, who received BM and PSC, respectively [1]. In this trial, a faster neutrophil and platelet engraftment were observed in PSC arm. However, there was no statistically significant difference for relapse and grade II–IV acute graft-versus-host disease (aGvHD) between groups. After 1 year of followup, chronic GVHD frequency was significantly higher in the PSC (65%) arm compared to BM (53%) arm.

Between 1998 and 2002, BM and PSC as a stem cell source were compared in 8 randomized trials [2–9]. Almost all of the patients included were diagnosed as leukemia. Number of patients included, remission status, conditioning regimen, GvHD prophylaxis, stem cell, and T-cell numbers were significantly different in these studies. Combined results suggest faster neutrophil and platelet engraftment with PSC compared to BM. One of the trials revealed similar grade II–IV aGVHD incidence. The largest randomized EBMT study has shown that the use of PSC significantly increased both the frequency of grade II–IV aGvHD (52%–39%; P: 0.013) and cGvHD (67%–54%; P: 0.0066) [5]. In EBMT trial omission of methotrexate on day 11 for aGVHD prophylaxis was suggested to be responsible for the increased aGVHD incidence; however, another meta-analysis was not able to verify this hypothesis [10]. Two out of four large-scale randomized trials showed increased chronic GVHD frequency (%22 and 13%) in patients treated with PSC [3, 5]. Other trials did not report statistically significant increase in chronic GVHD although an insignificant trend for increased cGvHD was observed [2, 4]. The long-term results of French [11] and EBMT [12] trials indicated a higher frequency of chronic

GVHD in the PSC compared to BM group; however, the short- and the long-term follow-up results of the North American [13] trial did not support these findings.

Graft versus tumor effect is mostly associated with the T lymphocytes. As the number of T lymphocytes is higher in PSC product compared to BM, we should expect a lower relapse rate in HSCT using PSC as stem cell source, whereas randomized trials do not report any decrease in relapse risk by using PSC.

Retrospective evaluation reveals lower transplant related mortality (TRM) in HSCT with PSC. IBMTR/EBMT results showed significant decrease in TRM with PSC in the advanced stage leukemia patients undergoing HSCT [1]. Randomized trials did not report statistically significant difference for TRM between PSC and BM. However, we cannot comment on the effect of disease subgroups, stages, and stem cell source which may have significant impact on TRM.

The most distinctive end point of HSCT is the overall survival. There are three big randomized trials reporting different results for this end point. EBMT [5] trial did not find any difference in terms of survival between BM and PSC; but another trial performed in the USA has reported a trend for increased 2-year overall survival (P: 0.06) for PBC [2]. After 30 months of followup, Canadians reported a %8 (P: 0.04) survival advantage in the PSC arm of the trial [14]. USA and Canadian trials in common indicated a survival advantage gained with PSC in advanced disease stages.

The meta-analysis of nine trials including a total of 1111 patients provides us very important data on this topic [15]. The meta-analysis confirms that the selection of PSC decreases the duration of neutrophil and platelet engraftment increases the frequency of grade III–IV aGVHD and chronic GVHD compared to BM. PSC use decreases the 3-year relapse rates in both early stage (%16–%20; P: 0.04) and advanced stage (%33–%51; P: 0.02) diseases compared to BM. On the other hand, PSC only increases the 3-year overall survival (%46–%31; P: 0.01) in the advanced stage disease group. It has been hard to generalize the results of these trials included in this meta-analysis because the majority of the patients had early stage disease such as chronic phase CML (75%) and AML in first complete remission.

Trials comparing PSC and BM as the stem cell source included almost only leukemia patients; on the other hand, very few patients diagnosed with lymphoma and myeloma have been included. Data on the stem cell source in HSCT setting for benign hematological diseases are not sufficient. EBMT has evaluated 692 severe aplastic anemia patients; over the age 20, there was no significant difference between PSC and BM in terms of cGVHD and mortality. In younger patients, cGVHD and mortality rates were higher (%27–%12) in the PSC group [16]. 5-year survival was increased %12 (%85–%73) when BM was preferred as the stem cell source in the subgroup of patients with the age ≤ 20. In conclusion, these data suggests that the type of the disease and the age of the patient play a role in deciding the optimal source of stem cell (Table 1).

Consequently, the randomized trials including adult patients point out that PSC increases chronic GVHD, provides overall survival advantage for advanced stage leukemia patients, but does not significantly have an effect on survival of early stage leukemia patients. On the other hand, in none of these trials, the followup duration has exceeded 3 years and the long-term results are still not known.

Table 1: Comparison of the stem cell sources.

	Cord Blood	PB	BM
Risk for the donor	None	Yes	Yes
Duration of searching (month)	≤1	3–6	3–6
Factors limiting the engraftment	Cell count	HLA match	HLA match
Dominant factor affecting the outcome	Engraftment failure delayed immune recovery	GVHD	GVHD
Minimal HLA match	4/6	9/10	9/10
Risk for GVHD	Low	High	High
Acute	Low	High	High
Chronic	Low	Higher	High
DLI possibility	None	Possible	Possible
Posttransplant infection risk	Higher	High	High
Immunotheraphy possibility	None	Yes	Yes

The joint IBMTR/EBMT study retrospectively evaluated a large patient population for the long-term results of PSC and BM as a stem cell source [17]. Between 1995-1996 patients over the age of 20 and with different stages of AML, ALL, and CML who underwent HSCT from PSC (n: 288) and BM (n: 462) has been analyzed. Follow-up data on 413 surviving patients (BM: 272; PSC: 141) has been evaluated and a median of six years followup results has been reported. Chronic GVHD incidence was 61% in PSC group and 45% in BM group. PSC has decreased TRM in the advanced stage AML and ALL subgroup but did not increase TRM in the chronic phase CML cases. For acute leukemia patients in first complete remission, there was no significant difference on survival according to stem cell source. But in patients, who achieved second complete remission, there was a trend for increased survival with use of PBC compared to BM (%49–%42). The effectiveness of stem cell source changes according to the stage in chronic leukemia patients. In chronic phase patients, BM provided 6-year survival advantage (%64–%43), but in the accelerated phase disease PSC seems to be superior in terms of survival (%33–%25).

Another trial from IBMTR included 773 (BM: 630; PSC: 143) acute leukemia patients 8–20 of age, who underwent HSCT from HLA match sibling donor between 1995–2000. The followup period was 4 years. Chronic GVHD frequency was 33% in PSC group and 19% in BM group. PSC has increased overall survival by %10 (%58–%48). There was no significant difference between stem cell sources in aGVHD and relapse rates.

CIMBTR data was analyzed for stem cell source of unrelated donors. This trial has evaluated 911 (PSC: 331; BM: 586) patients aged 18–60 years who were diagnosed as AML, ALL, CML, and MDS between 2000 and 2003. The frequency of aGVHD on posttransplantation day 100 (%58–%45) and chronic GVHD (%56–%42) were increased in significantly higher in the PSC group. Three years TRM (%45–%44) and the overall survival (%32–%30) showed no significant difference between PSC and BM groups.

2. The Role of Cord Blood

In theory, all of the patients who are candidates for HSCT but do not have a matched sibling donor but can provide adequate cord blood are candidates for HSCT with CB. HSCT with CB can be performed with 4/6 or 3/6 match, this is why %99 of all patients belonging to all ethnic groups can find acceptable CB units [17]. Therefore, CB is a very important stem cell source alternate but in adult patients stem cell number may be inadequate and there are disadvantages such as longer duration for engraftment and accompanying infections. HSCT with CB has been increased in last two decades; for adult patients, double CB transplantations have been performed successfully but there is no prospective randomized trial head to head comparing CB, PSC, and BM as a stem cell source.

IBMTR [18] and Eurocord [19] trials have retrospectively evaluated the CB use from sibling donors for pediatric patients; under the age of 15 and 1 year survival has been reported to be above 60%. Eurocord trial compared CB (n: 113) with BM (n: 2052). CB recipients were 3 years younger ($P < 0.001$), 9 kilograms lighter ($P < 0.001$), and were treated with lower doses of methotrexate (%28–%65; $P < 0.001$) for aGVHD prophylaxis. The median cell number was 4.7×10^7 nucleated cell/kg in CB recipients. Neutrophil and platelet engraftment ratios were lower with CB compared to BM. Grade II–IV aGVHD (%14–%24; $P = 0.02$) and chronic GVHD (%6–%15; $P = 0.02$) frequency were significantly lower in patients who received CB. There was no difference between groups in terms of 3 years overall survival (CB: %64; BM: %66). These data suggests that CB from HLA matched sibling has similar outcomes as BM from HLA match sister/brother for the pediatric group.

Unrelated CB transplantation data is mostly based on retrospective analysis. Two of three New York Blood Center studies are conducted in 0–11 years old children who were diagnosed with a hematological malignancy and 87% of all grafts were one or two antigen mismatched. The most important factor for the neutrophil engraftment in this series of 861 cases has been found to be the infused cell number. Neutrophil engraftment duration was median of 5 days earlier ($P = 0.0027$) in transplantations with HLA full matched compared to the HLA mismatched CB transplants [20]. HLA mismatch increases the risk of severe GVHD. Grade III–IV aGVHD rate was 8% with HLA A, B, DRB1 matched transplantations, but in mismatched cases it has been increased to 28% ($P = 0.006$). Multivariate analysis revealed that the most important markers for relapse were the stage of the disease and GVHD. The 3-year survival rates are predicted as 27% and 47% in hematological malignancies and genetic diseases, respectively.

In adult patients, CIBMTR/EBMT has retrospectively evaluated a total of 1525 patients who underwent unrelated HSCT for acute leukemia between 2002 and 2006 and randomized them into 3 different groups (CB: 165; BM: 472; PSC: 888) [21]. Disease-free survival ratios were similar between 8/8 and 7/8 HLA matched BM, PSC cases, and CB recipients. Considering that the 70% of CB group received two antigen mismatched transplants, this success of CB is remarkable. On the other hand, TRM was higher in the CB group when compared with 8/8 HLA matched PSC ($P = 0.003$) and BM ($P = 0.003$). Grade II–IV aGVHD ($P = 0.002$) and chronic GVHD ($P = 0.003$) frequency decreased with CB when compared to allele matched PSC; however, aGVHD ratios did not change when we compared the CB patients with 8/8 HLA matched BM recipients, but chronic GVHD frequency decreased in the CB group ($P = 0.01$).

Basic factors associated with the success of the HSCT with CB are cell number and the degree HLA match. The New York Blood Center (NYBC) has analyzed 910 CB transplantations and revealed that products with $\geq 5 \times 10^7$/kg cell count provided significantly higher 3-year survival rate [22]. The same data confirmed an absolute 3-year survival advantage of 25% with 6/6 HLA matched compared to 5/6 HLA matched CB HSCT. The joint CIBMTR/NYBC trial retrospectively evaluated 619 acute leukemia patients under age 16 during 1995–2003 period; 5-year survival ratios were higher with 6/6 HLA matched CB compared to 8/8 HLA matched BM transplantation (%63–%45). When there is one antigen mismatched CB, it is reasonable to increase the cell number ($>3 \times 10^7$/kg) with double donor to provide the same 5-year survival rate as HLA matched BM (%45) [23].

3. Conclusion

When we choose PSC instead of BM as the stem cell source, the following points should be beer in mind:

(i) chronic GvHD frequency increases,

(ii) in advanced stage leukemias TRM decreases,

(iii) in early phase CML cases TRM increases,

(iv) in advanced stage CML patients survival rate increases,

(v) survival in chronic phase CML cases decreases,

(vi) no effect can be achieved on relapse ratios,

(vii) no difference on disease-free survival and overall survival on acute leukemia,

(viii) aGVHD risk is similar with BM recipients in pediatric and adolescent acute leukemia patients, but chronic GVHD frequency is higher. Relapse ratios are similar in both BM and PSC groups. However, PSC increases TRM and overall mortality,

(ix) aGVHD frequency is similar in aplastic anemia patients. Mortality increases in the group of patients under the age of 20,

(x) acute and chronic GVHD frequency increases with unrelated transplantations. Survival rates are similar to BM recipients.

When we use CB for HSCT, the following points should be emphasized:

(i) An HSCT candidate, but who does not have HLA matched sister/brother and who can provide adequate cord blood for transplantation, can be a recipient.

(ii) The optimal graft selection procedure is still a matter of debate. The most important parameters are the number of nucleated cells and HLA match.

(iii) The success of the cord blood practice depends on the primary disease, conditioning regimen, defrosting the product and the experience of the HSCT center.

(iv) Especially when HLA mismatched unrelated and 5/6 HLA matched CB grafts are compared, CB can be a good alternative to unrelated transplantations.

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