



The extent of HLA class II allele level disparity in unrelated bone marrow transplantation: analysis of 1259 National Marrow Donor Program donor–recipient pairs

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Summary:

A comprehensive analysis of the HLA-D region loci, DRB1, DRB3, DRB5, DQA1, DQB1, DPA1 and DPB1, was performed to determine allelic diversity and underlying HLA disparity in 1259 bone marrow recipients and their unrelated donors transplanted through the National Marrow Donor Program. Although 43.0% of DRB1 alleles known to exist at the beginning of the study were found in this predominantly Caucasian transplant population, a few alleles predominated at each locus. In recipients, 67.1% of DRB1 alleles identified were one or two of six common DRB1 alleles. Only 118 (9.4%) donor–recipient pairs were matched for all alleles of DRB1, DQA1, DQB1, DPA1 and DPB1. While 79.4% of the pairs were matched for DRB1, only 13.2% were matched for DPB1 alleles. Almost 66% of pairs differed by more than one allele mismatch and 59.0% differed at more than one HLA-D locus. DQB1 was matched in 85.9% of DRB1-matched pairs. In contrast, only 13.9% of the pairs matched for DRB1, DQA1 and DQB1 were also matched for DPA1 and DPB1. This database, highlighting the underlying HLA disparity within the pairs, forms the foundation of an ongoing study to establish the relationship between HLA matching and successful outcome in unrelated allogeneic stem cell transplant. *Bone Marrow Transplantation* (2000) 25, 385–393.

Keywords: bone marrow transplantation; HLA matching

Transplantation of allogeneic hematopoietic stem cells has become an accepted treatment for many hematologic disorders. The optimal hematopoietic stem cell donor is an HLA-identical sibling because matching of major histocompatibility antigens significantly reduces the risks of transplant-related morbidity and mortality.^{1–5} Since approximately 65% of patients do not have a well-matched related donor, significant effort has been expended in developing DNA-based HLA typing methods to effectively identify an HLA matched unrelated donor to achieve a transplant outcome similar to that found with an HLA matched sibling donor.⁶

The loci encoding the HLA antigens are highly polymorphic and the probability of finding two unrelated individuals who share all of their HLA alleles is quite low.^{7–9} In the past, tests used for identification of an optimal allogeneic marrow donor included serologic typing of HLA-A, -B and -DR antigens as well as cellular assays of compatibility. It is now known that many of the serologically defined HLA specificities include several subtypes,¹⁰ and mismatches between alleles expressing the same serologic specificity can elicit deleterious immune responses.^{11,12} Today, DNA-based identification of HLA-A, -B and -DRB1 alleles has replaced the use of alternative histocompatibility testing methods.^{13,14} DNA-based typing of additional HLA loci (ie HLA-DQB1, -DPB1 and -C) is used by some centers to provide additional selection criteria.

Since 1987, the US National Marrow Donor Program (NMDP)¹⁵ has required a minimum five of six antigen-level match at HLA-A, -B and -DR. It is now possible to retrospectively identify the alleles present in stored NMDP patient and donor samples collected since 1988 using an identical level of typing resolution for all of the pairs. This report summarizes the results of comprehensive DNA-based testing for HLA class II alleles of 1259 unrelated donor–recipient pairs transplanted through the NMDP between 1988 and 1994 to determine the extent of HLA-D region disparity found within these pairs.

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Materials and methods

Paired samples selected

NMDP transplant and donor centers submit to the NMDP Research Sample Repository pretransplant whole blood samples from unrelated donors and their recipients. Cells from 1259 transplant pairs collected since 1988 were selected for study based on sample availability within the repository. The samples in the study represented 44.2% of patients transplanted through the NMDP during that time period (Table 1). A bias analysis was performed to determine if the transplant pairs included in the study were representative of all transplant pairs from that time period. The study was somewhat biased for disease ($P < 0.0001$), gender ($P = 0.008$), recipient race/ethnic group ($P = 0.04$), year of transplant ($P < 0.0001$), and transplant center ($P < 0.0001$). While many of the characteristics exhibiting bias are not likely to affect the conclusions of this study, differences in the ethnic mix of the patient populations served by transplant centers included or not included in the study will likely affect the allelic frequencies^{8,16} and the types of mismatches observed in the transplants under study.

At the time of the transplant, donors and recipients were,

Table 1 Donor and recipient characteristics

	Percentage ^a of study population	Percentage of total transplants in this study (No. in study/total No. transplants) ^b
Year of transplant		
1988	4.2	66.3 (53/80)
1989	6.2	44.1 (78/177)
1990	12.0	51.9 (151/291)
1991	19.1	53.0 (241/455)
1992	21.7	49.2 (273/555)
1993	22.9	40.4 (275/680)
1994	14.9	22.4 (188/840)
Diseases		
ALL	17.5	36.6 (219/599)
AML	14.6	35.1 (182/518)
CML	45.6	49.6 (570/1149)
Myelodysplastic disorders	7.7	41.9 (96/229)
Severe aplastic anemia	3.9	28.0 (49/175)
Other malignant diseases ^c	3.9	36.3 (49/135)
Other non-malignant diseases ^d	6.8	39.7 (85/214)
Race/Ethnic group		
	% Donors (No.)	% Recipients (No.) ^e
Asian/Pacific Islander	1.0 (12)	0.9 (11)
African American	1.0 (13)	2.6 (32)
Caucasian	87.8 (1105)	83.3 (1042)
Hispanic	2.2 (28)	4.1 (51)
Native American	0.7 (9)	0.7 (9)
Other/Unknown/Decline	7.3 (92)	8.4 (105)

^aNumbers in all charts may not add to 100% due to rounding.
^bFor example, in 1988, the NMDP facilitated 80 transplants. Donors and recipients from 53 of these transplants (66.3%) are included in this study.
^cIncludes lymphoma, other leukemias, and plasma cell disorders.
^dIncludes histiocytic disorders, inherited erythrocyte abnormalities, inherited immune system disorders, inherited metabolic disorders, and other non-malignant disorders.
^eNine recipients received marrow from two different donors.

at a minimum, characterized for HLA-A, HLA-B and HLA-DR antigens by various typing methods. In a significant number of cases, the donor–recipient pairs were also evaluated for compatibility using additional techniques such as MLC; however, this information was not collected by the NMDP and is not part of this study. Final donor selection was determined by each transplant center based on their own criteria and the minimum criterion established by the NMDP (5/6 antigen-level match at HLA-A, -B and -DR).

DNA-based typing

The DRB, DQA1, DQB1, DPA1, and DPB1 alleles of each sample were identified using locus-specific and/or one or more group-specific amplifications followed by hybridization with SSOP. DNA sequencing of DRB1 was also used as a primary method to identify alleles in approximately 1000 of these samples. Additional assays using sequence-specific amplification, restriction fragment length polymorphism analysis, and direct DNA sequencing of amplified DNA were used as needed in specific cases to aid in allele identification (Williams *et al*, in preparation; Schmeckpeper *et al*, in preparation). Each sample was typed at allele level by at least two independent typing laboratories in a blinded fashion. Discrepant typings were reviewed and, if required, further testing was used to identify the correct allele.

Interpretation of the typing results was based on the alleles described in the 1994 WHO HLA nomenclature report.¹⁷ Alleles differing only by silent substitutions were not distinguished. Alleles described (or deleted) since 1994, including new alleles identified in this study,¹⁸ were incorporated into the interpretation if a novel probe hybridization pattern or nucleotide sequence was obtained. DRB4 allele typing is not included here and will be described in a separate report (Schmeckpeper *et al*, in preparation). Samples with only a single allele identified by hybridization or sequence analysis were assumed to be homozygous after review of the complete typing for common haplotypic associations (eg DRB1*04/*07/*09 with DRB4), heterozygosity at other loci, and after review of transplant center typing.

Statistical analysis

Statistical tests applied to the data are described in the Results section.

Results

Donor and recipient characteristics

Class II HLA-D region alleles in 1259 unrelated donor-recipient paired samples from bone marrow transplants occurring from 1988 to 1994 were identified (Table 1). These samples were submitted from 62 transplant centers in the United States and 18 transplant centers in other countries. The majority of the transplant recipients (R) and donors (D) were Caucasian (R: 83.3%, D: 87.8%). In most of the transplants (76.6%), the patient and donor were from the same racial or ethnic group; 9.5% involved individuals

from different racial or ethnic groups and 13.9% involved patient and/or donor whose racial or ethnic background was not reported.

Allele frequency

Of the 107 DRB1 alleles described in the 1994 WHO nomenclature report¹⁷ (number excludes alleles which differ only by silent substitutions), 43 (40.2%) appeared in the recipient population and 42 (39.3%) in the donor population (Table 2). A similarly high percentage (52.8%) of the DPB1 alleles were found. In addition, five new DPB1 alleles, DPB1*5901, *6801, *7001, *7101, *7301, were identified in this sample set.¹⁸ A greater percentage of known alleles at other, less polymorphic loci appeared in the recipients or donors (DRB3: 4 observed/4 total known alleles; DRB5: 3/4; DQA1: 10/11; DQB1: 18/22; DPA1: 6/6).

In general, a few alleles accounted for the majority of the types identified at most of the loci. For DRB1, 67–68% of the alleles found in these samples were accounted for by one or two of the following six alleles, DRB1*0101, *0301, *0401, *0701, *1301 and *1501, each present at frequencies of at least 5%. DRB1*0301, *0701, *1501, each represented 14–15% of identified DRB1 alleles. Of the 10 DQA1 alleles observed in the transplant population, 59–60% of the identified alleles were accounted for by one or two of three alleles, DQA1*0102, *0201 and *0501, present at frequencies greater than 15%. Of the 18 DQB1 alleles observed in the transplant population, 78–79% of identified alleles were accounted for by one or two of six alleles, DQB1*0201, *0202, *0301, *0302, *0501, *0602, present at frequencies greater than 10%. A single allele, DPA1*0103, represented 81% of identified DPA1 alleles. Out of 33 DPB1 alleles observed, 77–78% of identified alleles were accounted for by one or two of four alleles, DPB1*0201, *0301, *0401, *0402, present at frequencies greater than 9–10%. A single allele, DPB1*0401, represented 43–46% of identified DPB1 alleles. These observations reflect the relatively homogeneous nature of this predominately Caucasian (eg R: 83.3%) transplant population. The most frequent alleles found in the recipients and donors are the alleles found in highest frequency in the US Caucasian population¹⁶ which most likely increased the availability of an HLA match.¹⁹

DRB1 matching

Almost 80% of the pairs were matched for both alleles at the DRB1 locus (Table 3). The relatively high level of DRB1 matching reflects the use of DR serologic and/or DNA-based typing in donor selection, the use of cellular assays to eliminate potential donors with HLA-D region differences, and the potential bias for selection of patients with common HLA haplotypes (ie patients with rare HLA haplotypes would have been unlikely to find donors during the time period evaluated, 1988–1994).

Of the 259 pairs with DRB1 mismatches, single allele mismatches were found in 218 (84.2%) pairs and both DRB1 alleles were mismatched in 41 (15.8%) pairs (Table 3). Ten of the single allele mismatches involved DRB1 homozygous donors who shared an allele with the

recipient (eg D: 1501, R: 1501,0103) and 12 involved DRB1 homozygous recipients who shared an allele with the donor (eg D: 0301,1401, R: 0301). Homozygosity in one member of the pair may affect the impact of the mismatch on the type of immune response generated (rejection/graft-versus-host disease).⁴

About 76% of the DRB1 mismatches involved alleles within an allele group (ie between alleles likely encoding molecules with the same serologic type) as defined by the first two numbers of the allele name (Figure 1). The remaining mismatches (23.7%) involved different allele groups (ie between alleles encoding molecules with different serologic types). The increased number of mismatches of DRB1*04, *11 and *13 compared to other DR groups reflects the frequency of these allele groups in the recipient population and the diversity within each of these allele groups. For example, DRB1*04 alleles represented 17.1% of the identified DRB1 alleles but this allele group contained 19 known alleles (DRB1*0401–DRB1*0419¹⁷). Several of these alleles appear at relatively high (>1%) frequencies in the transplant population (Table 2). The typing of DR4 by serology would not have identified these allele subtypes although the use of the MLC under optimal conditions¹⁴ or, for some transplants in more recent years, high resolution DNA-based testing should have detected the mismatch. In contrast, although DRB1*07 was present at high frequency (15.3%) in the transplant population, the limited allelic diversity in this group¹⁷ means that donors and recipients matched by serology for DR7 would also have been matched at the allele level.

Matching at other class II loci

Transplant centers are required to type for DR although some centers may have typed and matched for other HLA-D region loci (eg DQ). The level of allele matching for class II loci other than DRB1 varied from 87.2% matching for the second DRB locus (DRB3, DRB4, DRB5) to 13.2% matching at the DPB1 locus (Table 3). The difference in the degree of match between the closely linked loci, DPA1 (56.1%) and DPB1 (13.2%), most likely reflects the relatively limited number of DPA1 alleles ($n = 6$) compared to the extensive DPB1 diversity ($n = 53$) and the high frequency (80.8%) of a single DPA1 allele, DPA1*0103 (Table 2), in this predominantly Caucasian population. Since DQ and DP molecules are encoded by polymorphic A1 and B1 genes, mismatches at A1 or B1 loci result in a mismatch for the DQ or DP antigen. Of the 1259 pairs, 24.4% carry mismatches at DQA1 and/or DQB1 loci and 87.6% carry mismatches at DPA1 and/or DPB1 loci (Table 3).

Total level of HLA-D region allelic disparity in transplant pairs

One hundred and eighteen (9.4%) donor–recipient pairs were matched for all alleles of DRB1, DQA1, DQB1, DPA1 and DPB1 (Figure 2). All of the 118 pairs were matched for the second DRB locus (ie DRB3, DRB5), if present. A chi-square analysis indicated that no unique HLA characteristics were associated with the completely

Table 2 Class II allele frequencies^a

<i>DRB1</i> *	Donor frequency (%)	Recipient frequency (%)	<i>DQA1</i> *	Donor frequency (%)	Recipient frequency (%)	<i>DPA1</i> *	Donor frequency (%)	Recipient frequency (%)
0101	8.30	7.90	0101	9.81	9.81	0103	80.82	80.74
0102	1.43	1.43	0102	20.33	20.02	0104	0.71	0.52
0103	0.40	0.56	0103	6.63	6.67	0201	14.26	14.65
0301	14.14	14.06	0104	2.70	2.82	0202	3.93	3.61
0302	0.04	0.04	0201	15.33	15.13	0301	0.16	0.36
0401	9.81	9.85	0301	9.61	9.57	0401	0.12	0.12
0402	0.99	0.91	0302	8.70	8.82	<i>DPB1</i> *		
0403	0.60	0.64	0401	2.50	2.62	0101	6.27	5.96
0404	3.61	3.46	0501	24.23	24.27	0201	11.87	12.07
0405	0.24	0.40	0601	0.16	0.28	0202	0.79	0.44
0406	0.08	0.00	<i>DQB1</i> *			0301	9.17	10.21
0407	1.43	1.59	0201	14.26	14.10	0401	45.51	43.41
0408	0.32	0.16	0202	11.48	11.83	0402	10.84	11.99
0411	0.08	0.12	0301	17.00	17.00	0501	2.10	1.91
0701	15.29	15.25	0302	10.13	10.33	0601	1.83	1.39
0801	2.07	1.87	0303	4.73	4.65	0901	0.56	0.71
0802	0.16	0.44	0304	0.16	0.12	1001	1.31	1.51
0803	0.24	0.32	0305	0.08	0.08	1101	2.22	2.46
0804	0.20	0.12	0402	2.66	2.58	1301	1.31	1.55
0806	0.00	0.04	0501	10.60	10.76	1401	1.39	1.79
0811	0.12	0.12	0502	0.99	1.15	1501	0.79	0.68
0901	0.99	1.15	0503	1.99	2.03	1601	0.48	0.60
1001	0.71	0.79	0504	0.12	0.04	1701	1.67	1.51
1101	4.88	4.92	0601	1.07	0.91	1801	0.04	0.08
1102	0.12	0.24	0602	14.42	14.50	1901	0.68	0.20
1103	0.36	0.56	0603	5.88	5.92	2001	0.64	0.48
1104	2.82	2.42	0604	3.46	3.06	2101	0.04	0.04
1113	0.00	0.04	0608	0.00	0.04	2301	0.24	0.60
1124	0.04	0.00	0609	0.99	0.91	2701	0.00	0.08
1201	0.79	0.87				3401	0.04	0.00
1202	0.08	0.08				3501	0.00	0.08
1301	5.56	5.72				3901	0.04	0.08
1302	4.41	4.05				4501	0.08	0.00
1303	0.56	0.75				4601	0.00	0.04
1305	0.36	0.32				5201	0.00	0.04
1310	0.00	0.08				5901	0.04	0.00
1315	0.00	0.04				6801	0.00	0.04
1401	1.99	1.99				7001	0.00	0.04
1402	0.04	0.00				7101	0.00	0.04
1406	0.12	0.16				7301	0.04	0.00
1501	14.69	14.30						
1502	0.83	0.99						
1503	0.24	0.36						
1601	0.68	0.68						
1602	0.16	0.20						
<i>DRB3</i> ^{ab}								
3*0101	45.40	46.01						
3*0201	0.55	0.55						
3*0202	41.25	41.64						
3*0301	12.80	11.80						
<i>DRB5</i> *								
5*0101	90.19	89.66						
5*0102	5.02	5.05						
5*0202	4.78	5.29						

^aTable summarizes the allele frequencies of 1259 recipients and their donors. Frequency was defined as the number of times an allele appears divided by the total number of alleles (ie 2518 *DRB1* alleles total in the recipient pool). Samples which appeared to be homozygous for a particular allele were counted twice in this analysis. Nine recipients received marrow from two different donors and these recipients were counted twice in the frequency tables.

^bNot all individuals carry *DRB3* or *DRB5* loci. The allele frequencies are calculated only for those haplotypes that carry the locus based on known associations between *DRB1* and *DRB3* and between *DRB1* and *DRB5* loci. If an individual expressed a single allele at *DRB3*, for example, and the *DRB1* alleles were both expected to be associated with a *DRB3* locus, the individual was assumed to carry two identical *DRB3* alleles. *DRB4* alleles were not evaluated in this report.

Table 3 Summary of HLA-D region matches

Locus	No. of pairs typed	Matched at allele level (%) ^a	Mismatched at allele level		
			Total mismatches (%) ^b	1 Allele mismatched (% of total)	2 Alleles mismatched (% of total)
DRB1	1259	1000 (79.4)	259 (20.6)	218 (84.2)	41 (15.8)
DRB3/4/5	1235 ^c	1077 (87.2) ^d	158 (12.8)	ND ^e	ND
DQA1	1259	1052 (83.6)	207 (16.4)	189 (91.3)	18 (8.7)
DQB1	1259	965 (76.6)	294 (23.4)	272 (92.5)	22 (7.5)
DPA1	1259	706 (56.1)	553 (43.9)	505 (91.3)	48 (8.7)
DPB1	1259	166 (13.2)	1093 (86.8)	687 (62.9)	406 (37.1)

^aBoth alleles at the locus are identical in donor and recipient.
^bOne or both alleles are mismatched.
^cOf the 1259 pairs, only 1235 pairs had at least one DRB3, DRB4 or DRB5 allele in donor and/or recipient.
^dMatching was evaluated based on the number of loci and the alleles present, for example, D: DRB3*0101, blank was considered matched with R: DRB3*0101, DRB3*0101 but mismatched with R: DRB3*0101,*0201 or R: DRB5:0101, blank. DRB4 matching was evaluated based on the presence or absence of an amplified DRB4 allele. DRB4 disparity will be evaluated in a separate report (Schmeckpeper *et al*, in preparation).
^eND = not determined. Since DRB3, DRB4, and DRB5 alleles are not found on all haplotypes, the analysis of the number of alleles mismatched was not evaluated.

matched pairs. The remainder of the pairs had a variable degree of class II mismatch differing from a single allele mismatch at one locus ($n = 309$, 24.5%) to mismatches for two alleles at one locus ($n = 90$, 7.1%) to mismatches for nine alleles at all five loci ($n = 3$, 0.2%) (Figure 2). No pair differed for 10 alleles at all five loci, the maximum extent of variation possible. Approximately 66% of all pairs demonstrated a mismatch for two or more alleles at one or more loci including DRB1, DQA1, DQB1, DPA1 and DPB1, and 59.0% of all pairs were mismatched at more than one of these five loci. The majority of the mismatching was contributed by the DP loci. Within the 309 pairs which differed only by a single allele mismatch, most (280 pairs) differed only at the DPB1 locus and just 12 pairs differed only at the DRB1 locus (Table 4) (DRB3/DRB4/DRB5 matching was not included because not all haplotypes carry these loci).

Impact of matching one locus on matching at nearby loci

Because specific alleles at closely linked HLA loci tend to be associated at the population level (eg in linkage disequilibrium),^{9,20} the impact of matching at one HLA class II locus on the level of matching for other class II alleles was evaluated using a chi-square analysis. Overall, the matching at specific loci within the DR/DQ subregions was significantly correlated with matching at another locus in that region (Table 5). Thus, 92.8% of samples matched for DRB1 alleles were also matched for DQA1 alleles and 85.9% were matched for DQB1. Of the 965 pairs matched for DQB1, 98.7% were also matched for DQA1. Overall, 85.1% of all pairs were matched for DRB1, DQA1 and DQB1 loci. Since strong linkage disequilibrium is observed between specific DRB1 and DRB3/DRB4/DRB5 alleles and since the second expressed DRB locus is less polymorphic than the DRB1 locus, the level of matching of the second DRB locus among the DRB1 matched pairs was also analyzed. Matching for DRB3 alleles was high among DRB3 positive, DRB1 matched pairs; 87.5% were matched. Likewise, matching for DRB5 was high among DRB5 positive, DRB1 matched pairs; 99.4% were matched. Matching of DRB1 and DRB4 was not evaluated due to the lack of DRB4 allele level data.

DR/DQ matching did not result in matching at the DP loci (and vice versa) supporting the previous observations that recombination between the DR/DQ region and DP loci occurs at a rate of approximately 1% resulting in minimal linkage disequilibrium between DP and the rest of the HLA-D region.^{20,21} Since testing for DP differences was not routine in the unrelated donor match process and there is minimal linkage disequilibrium, there was not preferential selection of donors matched at these loci. Only 14.2% of pairs matched for DRB1 alleles were matched for DPB1 and only 14.6% of the pairs matched for DRB1, DQA1 and DQB1 alleles were also matched for DPB1 alleles. Analysis by odds ratios, however, did suggest that matching of DPA1 was correlated with matching at DPB1 and vice versa. Of the 166 pairs matched at DPB1, 94.0% were also matched for DPA1.

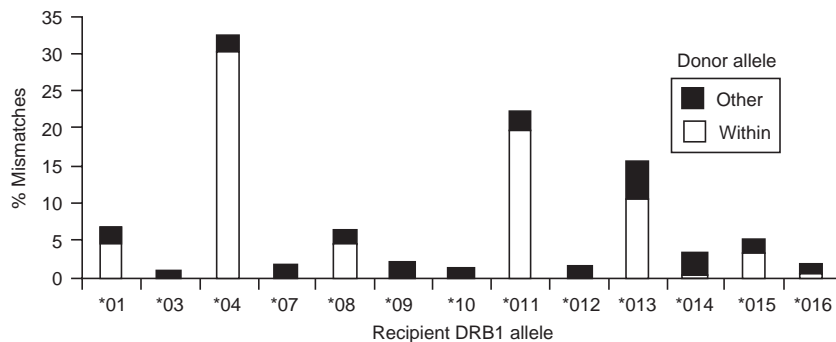


Figure 1 Categories of DRB1 mismatches. Categories of the kinds of DRB1 mismatches in the transplant pairs plotted as the percentage of the total DRB1 mismatches. Homozygous alleles which differed between donor and recipient were counted twice. Donor alleles labeled ‘within’ involve mismatches of donor alleles within the same allele group (eg R: 0401, D: 0402); ‘other’ indicates mismatches between allele groups (eg R: 0401, D: 0701).

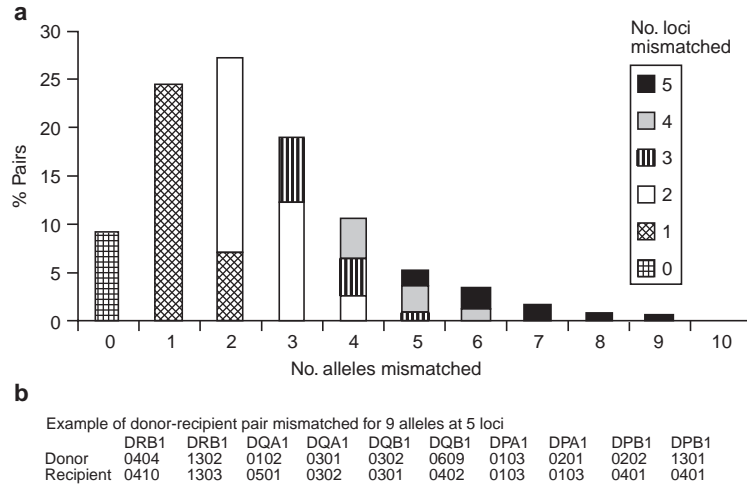


Figure 2 Disparity in donor-recipient pairs. (a) Percentage of donor-recipient pairs mismatched for none to 10 HLA-D region alleles at none to five loci (DRB1, DQA1, DQB1, DPA1, DPB1). Homozygous alleles which differed between donor and recipient were counted twice. Three pairs differed for nine alleles at all five loci. (b) The HLA-D region alleles carried by one of the three pairs.

Table 4 Transplant pairs differing only by a single allele mismatch (*n* = 309)

Locus with mismatch	No. of pairs (% of total)
DRB1	12 (3.9)
DQA1	1 (0.3)
DQB1	10 (3.2)
DPA1	6 (1.9)
DPB1	280 (90.6)

Table 5 Impact of matching on match at nearby loci

Pairs matched at loci (No. ^a)	Also matched at locus (No.; % ^b)
DRB1 (583 ^c)	DRB3 (510; 87.5)
DRB1 (330)	DRB5 (328; 99.4)
DRB1 (1000)	DQA1 (928; 92.8)
DRB1 (1000)	DQB1 (859; 85.9)
DRB1 (1000)	DPA1 (561; 56.1)
DRB1 (1000)	DPB1 (142; 14.2)
DRB1-DQA1 (928)	DQB1 (851; 91.7)
DRB1-DQB1 (859)	DQA1 (851; 99.1)
DQB1 (965)	DQA1 (952; 98.7)
DRB1-DQA1-DQB1 (851)	DPA1 (488; 57.3)
DRB1-DQA1-DQB1 (851)	DPB1 (124; 14.6)
DRB1-DQA1-DQB1-DPA1 (488)	DPB1 (118; 24.2)
DPB1 (166)	DPA1 (156; 94.0)

^aThe number of pairs matched at the indicated locus (loci). The relative gene order within the major histocompatibility complex: telomere-DRA-DRB3/4/5-DRB1-DQA1-DQB1-DPA1-DPB1-centromere.

^bThe number and percentage of pairs also matched for the indicated locus.

^c583 pairs that are matched at DRB1 also carry DRB3 alleles. Of these pairs, 510 or 87.5% were matched for both DRB1 and DRB3 alleles.

Common allele combinations of DR and DQ

The frequency with which specific DRB1 alleles were found with specific DQB1 alleles was evaluated for all DRB1 alleles with a frequency greater than or equal to 1%

Table 6 Common DRB1/DQB1 associations

DRB1 ^a	No. individuals with DRB1 allele	DQB1 ^b	Frequency of individuals with both DRB1 and DQB1 alleles (%) ^c
0101	392	0501	388 (99.0)
0102	72	0501	72 (100.0)
0301	664	0201	659 (99.2)
0401	475	0301	280 (58.9) ^d
0401	475	0302	236 (49.7)
0404	173	0302	171 (98.8)
0407	75	0301	62 (82.7) ^d
0701	717	0202	557 (77.7)
0701	717	0303	187 (26.1)
0801	99	0402	97 (98.0)
0901	52	0303	50 (96.2)
1101	236	0301	224 (94.9)
1104	128	0301	124 (96.9)
1301	281	0603	272 (96.8)
1302	206	0604	160 (77.7)
1302	206	0609	45 (21.8)
1401	100	0503	96 (96.0)
1501	684	0602	659 (96.3)

^aDRB1 alleles present at ≥1% in the donor-recipient population.

^bDQB1 allele found in linkage disequilibrium with the indicated DRB1 allele in Caucasians in other studies.^{9,16,22} Since family studies were not performed, it is not known if the two alleles are encoded by the same chromosome (*cis vs trans*).

^cFor example, of the 392 individuals who carried a DRB1*0101 allele, 388 or 99% also carried a DQB1*0501 allele.

^dIt is likely that some of these DQB1*0301 alleles are not on the same haplotype as DRB1*0401 or DRB1*0407. For example, 19 DRB1*0401 individuals carry both DQB1*0301 and DQB1*0302 alleles. These individuals also carry DRB1*1101 or DRB1*1104 alleles which are usually associated with DQB1*0301.

of the alleles of the study population (Table 6). For example, DRB1*0101 was found in 392 individuals (15.6% of 2518 total individuals); 388 (99.0%) of those individuals also carried a DQB1*0501 allele. While it is not known, in these individuals, if DQB1*0501 is encoded by the same

chromosome carrying DRB1*0101 (ie in a *cis* position), this frequent association coupled with family segregation data from many previous studies^{9,16,22} suggest that matching for DRB1*0101 will result in a match for DQB1*0501. The level of DQB1 matching varied with the DRB1 allele. DRB1*0701 was found associated with at least two different DQB1 alleles, DQB1*0202 (77.7% of individuals carrying DRB1*0701) and DQB1*0303 (26.1%). In this circumstance, DQB1 typing would be required to select the donor with the best DQB1 match. A similar reasoning applies to DRB1*0401 *0407 and *1302 which are also frequently associated with more than one DQB1 allele.

Haplotype matches

Of the 1259 pairs, 821 (65.2%) were matched for at least one allele at every locus. Since family studies were not carried out to confirm segregation of haplotypes, it is not known if these matches might be equivalent to a haplo-identical sibling match. The predicted HLA haplotypes of all of the donor–recipient pairs will be described in another study (Begovich, in preparation).

Discussion

One challenge in the use of unrelated *vs* related donors has been the increased potential for HLA allele level mismatches. While family members carry alleles of the HLA loci segregating within the family as linkage groups with limited possibility of recombination, the outbred human population carries a much more diverse collection of alleles on an array of haplotypes. This diversity is not fully evident using serologic-based testing but is revealed using the high resolution, allele level DNA-based methods employed in this study. This unrecognized disparity likely contributes to the observed differences in transplant morbidity and mortality rates between HLA-identical sibling transplants and HLA-matched unrelated donors in many studies.^{4,23–26}

In this study, the 1259 unrelated donor–recipient pairs were derived primarily from the Caucasian US population and exhibited a few predominant alleles at each locus. Since limited information exists on allele frequencies in large populations, it is not possible to compare frequencies of the transplant population with, for example, the US population. In spite of the apparent homogeneity in the pairs studied, only 9.4% of pairs were matched for five class II loci (DRB1, DQA1, DQB1, DPA1, DPB1) and the majority of pairs (70.5%) differed by one to three alleles affecting one to three of these loci. Pairs matched for DRB1 and both DQ loci represented 67.6% of the transplants evaluated. The DPB1 locus added the most extensive disparity among the pairs; 86.8% of the pairs were mismatched. Retrospective allele level class I typing of these samples is currently being analyzed; therefore, additional allelic mismatches at HLA-A, -B, -C loci are likely, further reducing the extent of HLA matching (Hurley *et al*, in preparation). Studies of the effect of HLA matching should take this extensive disparity at multiple loci into consideration in evaluation of the role of single HLA loci on outcome since the inevitable mismatches at other loci will likely have an impact on the

conclusions^{27,28} (Baxter-Lowe *et al*, in preparation). Extension of this analysis to transplant pairs from more genetically diverse racial or ethnic groups will likely identify an even greater level of disparity.

While the methodology to identify the full extent of HLA diversity now exists in the form of DNA-based techniques, the expense of extended HLA-D region typing make this characterization impractical for a transplant center that may have to HLA type multiple donors within a short period of time due to the patient's condition. Although the data are still limited, centers should focus their efforts on defining alleles at those loci which appear to be the most critical for transplant success.

Many transplant centers specify DRB1 allele matching in donor selection since studies on the impact of matching at individual HLA-D loci suggest that DRB1 identity reduces the risk of acute graft-versus-host disease and improves survival.^{1,11} As a result, the primary focus of HLA-D region typing is placed on identifying DRB1 alleles carried by donor and recipient.

A similar study²⁹ suggests that DQB1 may also encode a transplantation antigen and should be evaluated in selecting among several HLA-A, -B, -DRB1 matched donors. Strong linkage disequilibrium between DQB1 and DRB1 alleles should enhance the probability of finding a DRB1 and DQB1 matched donor. Since the allorecognition process likely detects the DQ $\alpha\beta$ heterodimer, alleles at both DQ loci must be matched to insure DQ identity. The data presented here show that matching at DRB1 and DQA1 matches DQB1 91.7% of the time, while matching for DRB1 and DQB1 matches DQA1 99.1% of the time. This suggests that typing can be focused on DQB1 in preference to DQA1 to select a donor who is potentially matched for DR and both DQ loci.

The impact of matching DRB3, DRB4 or DRB5 alleles is not known. The HLA molecules encoded by these loci do play a role in immune recognition^{30,31} but they are expressed at a lower level than DRB1-encoded molecules^{32–34} and may be a secondary alloreactive target. Since alleles at these loci are also in strong linkage disequilibrium with DRB1, DRB1 matched pairs sharing alleles at other expressed DRB loci should not be difficult to identify. Typing of DRB3, DRB4 and DRB5 loci is recommended to identify these matches when selecting among several HLA-A, -B, -DRB1 matched donors. The impact of DPB1 disparity is not yet known^{35,36} (Baxter-Lowe *et al*, in preparation).

Finally, selection of HLA class I matched donors is also important. The importance of matching at the antigen level for HLA-A and -B, at a minimum, and, more recently, for HLA-A, -B and -C alleles has been demonstrated.^{2,5,27,28,37} Because of the extensive HLA allele and haplotype diversity in the population, obtaining allele level matches for all HLA loci is difficult. The existence of extended haplotypes improves matching probabilities for some individuals since these haplotypes are found in most racial or ethnic groups.⁸

This study of the HLA class II alleles carried by 1259 unrelated donor–recipient pairs is the largest, most comprehensive analysis of HLA-D region disparity and matching in a transplant population to date. The study encompasses patients transplanted over 7 years from 80 transplant cen-

ters. The DNA-based typing carried out in a blinded duplicate approach, using a constant level of resolution and some of the most powerful methodologies for allele identification, ensures the consistency and reliability of this data set. The focus on defining only those allelic variations that result in differences in amino acid sequence between allelic products has identified those mismatches which might stimulate allorecognition. In contrast, earlier studies^{1,3,11,23,29,35} were often focused on one or a few HLA-D region loci and/or utilized less informative typing methodologies (eg serology) so that the underlying allelic disparity was not identified. The information provided in this retrospective study forms the foundation of a database to evaluate the role of HLA matching on bone marrow transplant outcome (Baxter-Lowe *et al*, in preparation; Petersdorf *et al*, in preparation), to develop strategies for permissive mismatches, and to enhance HLA typing strategies to select the optimal donor. These class II data will be complemented by typing data from ongoing studies to identify the class I alleles, HLA-A, -B and -C, carried by the same pairs (Hurley *et al*, in preparation). The large number of transplant pairs being characterized (1259) will improve the power of the statistical analysis, a limitation in many previous outcome studies. Continuing high resolution allele level HLA typing of more recent transplant pairs by the NMDP will continue to build this data set.

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