ASBMT American Society for Blood and Marrow Transplantation

Tumor Necrosis Factor Polymorphism Affects Transplantation Outcome in Patients with Myelodysplastic Syndrome but Not in Those with Chronic Myelogenous Leukemia, Independent of the Presence of HLA-DR15

Laura F. Newell,[1,2] Ted Gooley,[1,3] John A. Hansen,[1,2] Derek L. Stirewalt,[1,2] Effie W. Petersdorf,[1,2] H. Joachim Deeg[1,2]

Both the presence of HLA-DR15 and tumor necrosis factor (TNF)-α levels have been reported to affect outcome after hematopoietic cell transplantation (HCT). Patients with a myelodysplastic syndrome (MDS) show a high prevalence of HLA-DR15 and express high levels of TNF-α in the bone marrow. The present analysis involving 7950 patients showed an HLA-DR15 frequency of 31% in patients with MDS, compared with only 23% in patients with chronic myelogenous leukemia (CML). HLA-DR15 was more prevalent in Caucasian patients than in non-Caucasian patients ($P = .01$). The numbers of patients in the non-Caucasian subgroups were too small to allow further analysis. Among Caucasian patients with MDS and CML, the presence of HLA-DR15 did not significantly affect the occurrence of graft-versus-host disease, relapse, nonrelapse mortality (NRM), or survival. However, there was a significant correlation between DR15 and TNF polymorphisms at position -308 among patients with MDS, and the TNF-308 AG genotype conferred an increased risk of NRM compared with the GG genotype (hazard ratio [HR], 1.49; $P = .02$), even after adjusting for DR15. Conversely, the TNF-863 AA genotype was correlated with decreased overall mortality and NRM compared with the CC genotype (HR, 0.36, $P = .04$ vs HR, 0.13, $P = .04$), even after adjusting for DR15. There was no significant association between TNF-308 or -863 polymorphisms and transplantation outcome in CML patients. These results suggest that TNF polymorphisms, but not DR15, affect transplantation outcome in a disease-dependent manner.

Biol Blood Marrow Transplant 16: 1700-1706 (2010) © *2010 American Society for Blood and Marrow Transplantation*

KEY WORDS: Hematopoietic cell transplantation, MDS, TNFx, Non-relapse mortality

INTRODUCTION

Major histocompatibility complex (MHC) class II genes (HLA-DR, -DQ, and -DP in humans) were originally described in the mouse as immune response genes [1]. Subsequently, many examples of disease association with class II HLA antigens/alleles in humans have been recognized. In the setting of hematopoietic cell transplantation (HCT), the presence of the class II antigen HLA-DR2 (subsequently split into DR15 and DR16) was reported to affect transplantation outcome, particularly the occurrence of graft-versus-host disease (GVHD) and survival [2-4]. Centromeric to the HLA gene complex on chromosome 6, and closely linked to HLA-DR, is the gene encoding the proinflammatory cytokine tumor necrosis factor (TNF)-α, which is also thought to play a role in transplantation outcome [5,6]. As we and others have shown, significant upregulation of TNF in the marrow of patients with a myelodysplastic syndrome (MDS) and HLA-DR15 (DR2) have been associated with MDS [7-10]. We were interested in investigating whether transplantation outcome in patients with MDS is dependent on the presence of HLA-DR15, TNF genotype, or both.

TNF shows single nucleotide polymorphisms (SNPs) at positions -308 and -863. The TNF-308

From the [1]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington; [2]Department of Medicine; and [3] Biostatistics, University of Washington School of Medicine, Seattle, Washington.

Financial disclosure: See Acknowledgments, page 1705.

Correspondence and reprint requests: H. Joachim Deeg, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N, D1-100, Seattle, WA 98109 (e-mail: jdeeg@fhcrc.org).

Received April 6, 2010; accepted June 1, 2010

1083-8791/$36.00
doi:10.1016/j.bbmt.2010.06.003

position consists of a guanine (G) or an adenine nucleotide (A). The TNF-308 GG genotype has been shown to result in higher TNF levels compared with the AA genotype [11]. Kroeger et al. [12] reported that the -308 polymorphism altered the affinity of nuclear factor binding and differentially affected transcription of the TNF-α gene; the presence of an adenine nucleotide at -308 increased transcription at least 2-fold above the levels seen with guanine. The TNF-863 cytosin (C) and adenine (A) polymorphism, which has been implicated in several autoimmune diseases [13,14], has been speculated to influence TNF-α expression through differential binding of nuclear factor-κB (NF-κB) complexes and through allele-specific chromatin remodeling [15]. The -863 A allele ultimately is associated with increased TNF-α production [16].

In the present study, we first analyzed the prevalence of HLA-DR15 in 7950 patients who underwent HCT for various hematologic disorders, including MDS. Our findings confirmed a high prevalence in patients with MDS. We then selected the cohort of patients with the lowest prevalence of HLA-DR15, those with chronic myelogenous leukemia (CML), as a comparison group. We used these two cohorts to evaluate linkage disequilibrium between HLA-DR15 and the TNF-308 and -863 SNPs in our patient population, and also to analyze the effects of HLA-DR15 alleles and TNF polymorphisms on transplantation outcome.

METHODS

Patient, Disease, and Transplant Characteristics

This retrospective analysis involved 7950 patients undergoing a first HCT at the Fred Hutchinson Cancer Research Center for aplastic anemia, acute myelogenous and lymphoblastic leukemias, chronic lymphocytic leukemia, CML, Hodgkin disease, multiple myeloma, non-Hodgkin lymphoma, or MDS. Details of the conditioning regimens; GVHD diagnosis, prophylaxis, and therapy; infection prophylaxis; supportive care measures; and assessment of relapse have been reported elsewhere [17-22]. All patients provided informed consent for ongoing research studies in accordance with the requirements of Fred Hutchinson Cancer Research Center's Institutional Review Board.

HLA Typing

Related donors were HLA-identical siblings or mismatched relatives as determined by family study before HCT [23]. Unrelated donors were characterized for HLA-A, -B, -C, -DRB1, and -DQB1 alleles using sequence-specific oligonucleotide probe hybridization or sequencing methods [23]. Patients who expressed at least one copy of a DR15 allele were classified as DR15-positive.

Genotyping of TNF SNPs

Genotyping of the SNPs of TNF-α involving positions -308 and -863 was carried out by a multiplex polymerase chain reaction (PCR) assay, as described previously [24]. No TNF typing was done besides that performed for a previous study [24].

Statistical Analysis

The χ^2 test was used to compare the frequency of DR15 across various diagnoses, as well as the association between the presence of DR15 and the TNF genotype. Cox regression was used to examine the association between both the presence of DR15 and TNF genotype and the time-to-event outcomes overall mortality, relapse, and nonrelapse mortality (NRM). Logistic regression was used to examine the same associations with the outcome of grade II-IV acute GVHD (aGVHD). Regression models were adjusted for patient age, disease severity (high vs low), and type of donor (HLA-identical sibling vs others). Given the linkage disequilibrium between DR15 and TNF, both DR15 and each of the TNF polymorphisms were included in all regression models, to evaluate the association of one after adjustment for the others. Two-sided P values from regression models were estimated from the Wald test; no adjustments were made for multiple comparisons [25].

RESULTS

Frequencies of HLA-DR15 and TNF Polymorphisms Depend on Diagnosis and Racial Origin

A total of 6866 Caucasian and 1084 known non-Caucasian patients undergoing first HCT at our center had HLA data available that allowed categorization in terms of expressing or not expressing at least one copy of a DR15 allele. The frequency of DR15 was higher in Caucasians than in non-Caucasians (25.8% vs 22.3%; $P = .01$). Among 765 Caucasian patients with a diagnosis of MDS, 237 (31.0%) had at least one DR15 allele, compared with 393 of 1644 patients with CML (23.9%). The frequencies of other diagnoses are listed in Table 1. A global test of the equality of the proportion of patients positive for DR15 across all diseases yielded $P = .001$, supporting the hypothesis that the prevalence of DR15 differs significantly depending on the diagnosis. Characteristics of the patients with MDS or CML are summarized in Table 2.

The frequency of the TNF-308 genotypes differed between Caucasians and non-Caucasians. Specifically, the GG genotype occurred in 68.5% of Caucasians

Table 1. Incidence of the DRB15 Allele by Disease Category in Caucasian Patients**

Disease	Number of Patients with ≥ 1 Copy of a DRB15** Allele/Total Number	Percent DRB 15-Positive
Aplastic anemia	84/325	25.8
ALL	237/993	23.9
Acute myelogenous leukemia	481/1907	25.2
Chronic lymphocytic leukemia	28/97	28.9
CML	393/1644	23.9
Hodgkin disease	57/162	35.2
Multiple myeloma	98/343	28.6
Non-Hodgkin lymphoma	158/630	25.1
MDS	237/765	31.0

ALL indicates acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome.

and in 85.9% of non-Caucasians; the AG genotype was present in 29.2% of Caucasians and in 13.0% of non-Caucasians, and the AA genotype was present in 2.6% of Caucasians and in 1.1% of non-Caucasians ($P < .0001$). The differences in frequency of the TNF-863 genotypes were not as marked, but were still statistically significant ($P = .02$). Based on these differences, we restricted the subsequent analyses to the Caucasian population. Table 3 summarizes the allele and genotype frequencies for the TNF-308 and -863

Table 2. Patient, Disease, and Transplant Characteristics of Caucasian Patients with MDS or CML

	MDS	CML
Number of patients	765	1644
Age, years, median (range)	48.3 (0.9-78.9)	37.1 (0.7-69.7)
Patient-donor sex match, n (%)		
Female-female	150 (20)	307 (19)
Female-male	168 (22)	343 (21)
Male-female	186 (24)	401 (24)
Male-male	253 (33)	588 (36)
Unknown	8 (1)	5 (<1)
Conditioning regimen, n (%)*		
High intensity	706 (92)	1626 (99)
Reduced intensity	59 (8)	18 (1)
Donor, n (%)		
HLA-identical sibling	346 (45)	731 (44)
HLA-matched unrelated donor	349 (46)	676 (41)
Nonsibling relative or mismatched sibling	62 (8)	235 (14)
Autologous†	8 (1)	2 (<1)
Stem cell source, n (%)		
Bone marrow	403 (53)	1522 (93)
Peripheral blood	356 (47)	117 (7)
Bone marrow and peripheral blood	4 (<1)	2 (<1)
Umbilical cord blood	2 (<1)	3 (<1)
Period of transplantation, n (%)		
1969-1985	26 (3)	228 (14)
1986-1990	75 (10)	391 (24)
1991-1995	162 (21)	490 (30)
1996-2000	173 (23)	393 (24)
2001-2005	224 (29)	113 (7)
2006-present	105 (14)	29 (2)

MDS indicates myelodysplastic syndrome; CML, chronic myelogenous leukemia.

*High intensity: cyclophosphamide (Cy)/total body irradiation (TBI), busulfan (B)/Cy, and fludarabine (Flu)/Bu [31-34]; reduced intensity: Flu/TBI [35].

†Not considered further in the outcome analysis.

Table 3. Allele and Genotype Frequencies of TNF-308 and -863 SNPs and Association between HLA-DR15 and TNF SNPs in Caucasian Patients with MDS or CML

	MDS	CML	χ^2 *P* Values for MDS and CML
TNF-308 alleles*			
A	120/375 (32%)	181/593 (30.5%)	
G	366/375 (97.6%)	577/593 (97.3%)	
Genotypes			
AA	9/375 (2.4%)	16/593 (2.7%)	
AG	111/375 (29.6%)	165/593 (27.8%)	
GG	255/375 (68%)	412/593 (69.5%)	
TNF-863 alleles*			
A	115/372 (30.7%)	165/593 (27.8%)	
C	358/372 (95.5%)	582/593 (98.1%)	
Genotypes			
AA	14/372 (3.8%)	11/593 (1.9%)	
AC	101/372 (27.2%)	154/593 (26.0%)	
CC	257/372 (69.1%)	428/593 (72.2%)	
Proportion of DR15-positive patients			
TNF-308			$P = .003$, $P = .54$
AA	0/9	2/16 (12.5%)	
AG	26/111 (23.4%)	37/165 (22.4%)	
GG	97/255 (38.0%)	99/412 (24.0%)	
TNF-863			$P = .11$, $P = .03$
AA	5/14 (35.7%)	0/11	
AC	25/101 (24.8%)	28/154 (18.2%)	
CC	93/257 (36.2%)	110/428 (25.7%)	

SNP indicates single nucleotide polymorphism; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; MDS, myelodysplastic syndrome.

*Heterozygotes contribute to both allele categories.

SNPs in Caucasians, along with the correlation with DR15, which was significant for TNF-308 ($P = .003$). The number of patients in any particular non-Caucasian population was not sufficiently large to allow separate analyses.

DR15 and TNF-308 and -863 Polymorphisms in MDS and CML

Table 3 summarizes the associations between SNP genotypes and DR15. The frequency of DR15 differed across TNF-308 genotypes in patients with MDS ($P = .003$), and the presence of at least one DR15 allele was suggestively (but not statistically significantly) different across TNF-863 genotypes ($P = .11$). In patients with CML, the presence of DR15 was not statistically significantly correlated with the TNF-308 genotype ($P = .54$), whereas it was correlated with the TNF-863 genotype ($P = .03$).

Associations of DR15 Positivity and TNF-308 and -863 SNPs with Transplantation Outcome in MDS Patients

After adjusting for TNF-863 and the presence of DR15, the TNF-308 AG genotype in patients with MDS was associated with an increased risk of NRM relative to the GG genotype. Overall mortality was also increased, but not significantly so (Table 4). The AA genotype also tended to be associated with increased failure rate relative to the GG genotype for

Table 4. Association of DR15 and TNF-308 and -863 SNPs with Transplantation Outcome in Caucasian Patients with MDS

Determinant	Overall Mortality	Relapse	NRM	aGVHD Grade II-IV
TNF-308				
GG	1	1	1	1
AG	1.20 (0.90-1.62); $P = .21$	0.76 (0.43-1.35); $P = .35$	1.41 (1.01-1.98); $P = .05$	1.46 (0.82-2.59); $P = .21$
AA	1.57 (0.72-3.42); $P = .26$	1.99 (0.59-6.70); $P = .27$	1.21 (0.44-3.37); $P = .71$	0.69 (0.15-3.14); $P = .47$
TNF-863				
CC	1	1	1	1
AC	1.19 (0.88-1.61); $P = .26$	1.74 (1.05-2.87); $P = .03$	1.12 (0.78-1.60); $P = .55$	0.79 (0.45-1.37); $P = .97$
AA	0.39 (0.14-1.06); $P = .06$	1.24 (0.44-3.52); $P = .69$	0.15 (0.02-1.05); $P = .06$	0.64 (0.19-2.09); $P = .58$
DR15				
Absent	1	1	1	1
Present	1.03 (0.77-1.38); $P = .84$	0.89 (0.53-1.49); $P = .66$	1.00 (0.71-1.42); $P = .99$	1.29 (0.76-2.21); $P = .35$

SNP indicates single nucleotide polymorphism. Values listed are HR and 95% CI for overall mortality, relapse, and NRM and OR and 95% CI for GVHD.

each of the foregoing endpoints; however, none of these associations was statistically significant (Table 4), because there were only 9 patients with this genotype. No statistically significant association was found between TNF-308 SNPs and relapse (Table 4). Patients with the TNF-863 AC genotype had similar overall mortality and NRM as those with the CC genotype (Table 4), whereas patients with the AA genotype had lower overall mortality and NRM compared with those with the CC genotype. The small number of patients with the AA genotype (n = 14) mandates a cautious interpretation of these results, however. The associations between TNF and overall mortality, NRM, and relapse did not appear to depend on the presence of DR15 (with interaction *P* values ranging from .39 to .95). Moreover, the magnitudes of the associations between TNF and each of these outcomes after adjusting for the presence of DR15 were virtually the same as the magnitudes of associations between TNF and outcomes without adjusting for the presence of DR15.

No statistically significant association was found between TNF-863 SNPs and aGVHD (Table 4). The impact of the presence of TNF-308 SNPs on grade II-IV aGVHD was dependent on DR15 status ($P = .04$, test of interaction). In DR15-negative patients, the TNF-308 AG genotype was associated with an increased risk of aGVHD compared to patients with the GG genotype (odds ratio [OR], 2.25; 95% confidence interval [CI], 1.12-4.49; $P = .02$); however, the association of the TNF-308 AG genotype with aGVHD in DR15-positive patients was in the opposite direction (OR, 0.37; 95% CI, 0.12-1.11; $P = .08$). Furthermore, no statistically significant association was noted between the presence of an HLA-DR15 allele per se and transplantation outcome (i.e., overall mortality, relapse, NRM, or grade II-IV aGVHD) (Table 4).

Association of DR15 Positivity and TNF-308 and -863 SNPs with Outcome in CML Patients

Among CML patients, no statistically significant associations were found among TNF-308 or -863 SNPs and overall mortality, relapse, NRM, and grade II-IV aGVHD. In addition, there was no statistically significant association between the presence of a DR15 allele and outcome (Table 5).

HLA-B, TNF Polymorphisms, and Transplantation Outcome

Given the location of the TNF gene within 200 kb of HLA-B, we also evaluated possible associations between HLA-B and TNF. Several HLA-B antigens showed an association with the TNF SNPs examined, but none of the associations between TNF and transplantation outcome was qualitatively changed after adjustments were made for the presence of these HLA-B antigens (data not shown).

DISCUSSION

The present analysis of data from patients undergoing HCT for lymphoid or hematologic disorders shows significant disease-dependent differences in the frequency of the presence of HLA-DR15, with at least one DR15 allele found in 31% of patients with MDS and 23% of those with CML. A second analysis, restricted to Caucasian patients with MDS or CML for whom molecular information on TNF polymorphisms was available, showed a statistically significant correlation of the TNF-308 genotype with DR15 in MDS patients, but not in CML patients. The TNF-863 genotype showed a suggestive correlation with DR15 in MDS patients and a statistically significant correlation in CML patients. In patients with MDS, the TNF-308 AG genotype was associated with an increased risk of NRM relative to the GG genotype, whereas, conversely, the -863 AA genotype was associated with a suggestively decreased risk of overall mortality and NRM relative to the CC genotype. These results must be interpreted with caution, however, because of the small number of patients with the TNF-863 AA genotype. Importantly, these associations were seen after adjusting for the presence of DR15 alleles, suggesting that the presence or absence of DR15 did not explain the association of the TNF SNPs with transplantation outcome. In contrast to

Table 5. Lack of Association of DR15 and TNF-308 and -863 SNPs with Outcome in Caucasian Patients with CML

Determinant	Overall Mortality	Relapse	NRM	aGVHD Grade II-IV
TNF-308				
GG	1	1	1	1
AG	0.97 (0.74-1.28); $P = .85$	1.31 (0.89-1.91); $P = .17$	0.99 (0.71-1.36); $P = .93$	0.82 (0.51-1.33); $P = .43$
AA	0.85 (0.40-1.83); $P = .68$	0.85 (0.27-2.74); $P = .79$	0.82 (0.33-2.01); $P = .82$	0.86 (0.24-3.05); $P = .81$
TNF-863				
CC	1	1	1	1
AC	1.06 (0.81-1.40); $P = .67$	1.32 (0.90-1.94); $P = .16$	0.97 (0.70-1.36); $P = .87$	0.86 (0.53-1.41); $P = .56$
AA	1.14 (0.53-2.45); $P = .74$	0.89 (0.21-3.66); $P = .87$	1.06 (0.43-2.65); $P = .89$	0.52 (0.12-2.32); $P = .39$
DR15				
Absent	1	1	1	1
Present	0.92 (0.68-1.23); $P = .55$	0.98 (0.65-1.48); $P = .91$	0.92 (0.65-1.31); $P = .65$	1.14 (0.68-1.89); $P = .63$

Values listed are HR (95% CI) for overall mortality, relapse, and NRM and OR (95% CI) for GVHD.

the MDS cohort, the patients with CML exhibited no statistically significant effects of TNF-308 and -863 SNPs on transplantation outcome. Neither the MDS nor the CML cohort demonstrated any statistically significant associations between DR15 itself and transplantation outcome.

Our results are in contrast to those in some previous reports that showed correlations between DR15 and transplantation outcome in patients with various diagnoses [2-4]. For example, Stern et al. [4] observed a significantly higher 5-year survival rate in DR15-positive patients compared with DR15-negative patients (76% vs 55%; $P = .04$), along with a lower 5-year probability of disease-related mortality in the DR15-positive patients (5% vs 24%; $P = .02$). Similar data were reported by Battiwalla et al. [2], who found a significantly lower incidence of aGVHD in DR15-positive patients than in DR15-negative patients (23% vs 42%; $P = .041$). However, that analysis included only 20 patients with MDS, and patients with serologically determined DR2 (who had not been allele-typed) were included in the DR15-negative group. In the study by Davidson et al. [3], DR15-positive adult patients demonstrated improved day 100 survival ($P = .03$) and overall survival ($P = .0143$). Moreover, in patients who developed aGVHD, survival was superior in those who were DR15-positive ($P = .02$). However, an analysis of results in 88 patients undergoing HCT from HLA-identical unrelated donors showed inferior survival with DR15 positivity ($P = .02$), and DR15-negativity was associated with improved survival in patients who developed aGVHD ($P = .009$).

In the present study, we found no correlation between the presence or absence of HLA-DR15 and transplantation outcomes, including GVHD, relapse, NRM, and survival. Although it is conceivable that differences in results among reports are related to differing compositions of the patient cohorts studied, we found no correlation of DR15 with outcome in patients with either MDS or CML undergoing transplantation from either related or unrelated donors. Similarly, we noted no associations between specific HLA-B alleles and transplantation outcome. In fact, our data suggest correlations with TNF polymorphisms, particularly the TNF–308 G allele. Of course, although 91% of the patients with MDS and 85% of those with CML had an HLA-matched donor (including HLA-DR), TNF polymorphism data were obtained only from patients, and these data might have differed from those for the donors, particularly in the unrelated donor setting.

Lin et al. [24] analyzed data from 570 patients who underwent HCT at our center and their HLA-identical sibling donors for an association between GVHD and cytokine SNPs [24]. They analyzed 7 different SNPs in 5 cytokine genes, including TNF-308, and found no statistically significant association between aGVHD and the TNF-308 genotype. That study included patients with various diagnoses, however, and in view of the differences among different diagnostic groups observed in the present analysis, the data cannot be globally extrapolated. Middleton et al. [26] determined TNF and interleukin-10 polymorphic allele frequencies in 80 patients with ALL or CML. They then correlated the polymorphism results with the incidence and severity of GVHD in 49 patients who subsequently underwent HCT from an HLA-identical sibling donor. They found no significant association of outcome with the TNF-308 polymorphism, although they did observe an association of GVHD with a microsatellite near the TNF locus. Takahashi et al. [27] reported a significant association between the donor TNF-308 SNP (which was not determined in the present study) and the severity of aGVHD ($P = .04$) in patients undergoing transplantation primarily from HLA-identical related donors. Although patient TNF-308 polymorphism pretransplantation was not associated with aGVHD, there was a significant association with polymorphism in postengraftment samples ($P = .04$), suggesting a relationship to donor cell polymorphism. In yet another analysis of results in 77 patients undergoing transplantation from HLA-identical sibling donors, Bertinetto et al. [28] observed significant associations between grade II-IV GVHD and TNF-308 or -863 polymorphisms. Finally, Shaw et al. [29] reported delayed neutrophil engraftment in

patients with CML, acute leukemia, or other malignant disease undergoing transplantation from a related or an unrelated donor if the TNF-308 AG genotype was present in either the patient ($P = .03$) or the donor ($P = .02$). No such correlation was found in the present study (data not shown).

Although both TNF-308 and -863 SNPs are thought to be associated with increased levels of TNF-α production, the present analysis showed quite different effects on post-HCT outcomes in MDS patients. Patients with the -308 AG genotype had an increased risk of NRM relative to those with the GG genotype, whereas patients with the -863 AA genotype had suggestively decreased risks of overall mortality and NRM relative to the CC genotype. Of course, our analysis, much like other reports, did not include the study of TNF-α protein levels in either blood or marrow [30], which would be desirable to more firmly establish a role of the gene product in transplantation outcome.

As far as a potential impact of HLA-DR15 on treatment outcome is concerned, several hypotheses have been proposed. It has been suggested that the molecule preferentially presents autoantigens on hematopoietic precursor cells, thereby evoking an immune reaction of T cells [4,7]. It also has been suggested that DR15 is associated with an immune profile that increases the responsiveness to immunosuppressive therapy [2]. In view of the present results, which suggest that transplantation outcome was affected primarily by TNF polymorphisms (in linkage disequilibrium with HLA-DR alleles) rather than by DR alleles themselves, focusing future studies on cytokine profiles [24,26,27] might prove more productive.

In summary, in the present study we found significant differences in the frequency of HLA-DR15 in patients with different hematologic diagnoses who were referred for HCT. The data in Caucasian patients showed an association of DR15 with TNF-308 and -863 polymorphisms and an effect of these polymorphisms on transplantation outcome in patients with MDS, but not in a large comparison group of patients with CML. The data suggest that previously reported correlations of transplant outcome with DR15 might in fact be correlations with TNF polymorphisms. As ongoing clinical studies are exploring the effect of TNF blockade on transplantation outcomes, examining whether therapeutic results are correlated with TNF genotype might be of interest.

ACKNOWLEDGMENTS

The authors thank Bonnie Larson and Helen Crawford for helping with manuscript preparation, and Gary Schoch and Anajane Smith for updating the HLA typing information and clinical outcome data.

Financial disclosure: This work was supported in part by National Institutes of Health Grants HL036444, CA18029, AI33484, and CA15704.

REFERENCES

1. Klein J, Gutknecht J, Fischer N. The major histocompatibility complex and human evolution (review). *Trends Genet*. 1990;6: 7-11.
2. Battiwalla M, Hahn T, Radovic M, et al. Human leukocyte antigen (HLA) DR15 is associated with reduced incidence of acute GVHD in HLA-matched allogeneic transplantation but does not impact chronic GVHD incidence. *Blood*. 2006;107:1970-1973.
3. Davidson JA, Tate DG, Poulton KV, et al. HLA-DR15, reduced relapse rate and improved survival after HLA identical sibling hemopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:493-494.
4. Stern M, Passweg J, Tiercy JM, et al. Human leukocyte antigen DR15 is associated with reduced relapse rate and improved survival after human leukocyte antigen–identical sibling hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2006;12:1169-1175.
5. Holler E, Kolb HJ, Möller A, et al. Increased serum levels of tumor necrosis factor α precede major complications of bone marrow transplantation. *Blood*. 1990;75:1011-1016.
6. Wolff D, Roessler V, Steiner B, et al. Treatment of steroid-resistant acute graft-versus-host disease with daclizumab and etanercept. *Bone Marrow Transplant*. 2005;35:1003-1010.
7. Sauntharajah Y, Nakamura R, Nam JM, et al. HLA-DR15 (DR2) is overrepresented in myelodysplastic syndrome and aplastic anemia and predicts a response to immunosuppression in myelodysplastic syndrome. *Blood*. 2002;100:1570-1574.
8. Sugimori C, Yamazaki H, Feng X, et al. Roles of DRB1*1501 and DRB1*1502 in the pathogenesis of aplastic anemia. *Exp Hematol*. 2007;35:13-20.
9. Xiao M, Qiong L, Yan Z, et al. Experimental and clinical characteristics in myelodysplastic syndrome patients with or without the HLA-DR I 5 allele. *Hematol Oncol*. 2010;28:98-103.
10. Maciejewski JP, Follmann D, Nakamura R, et al. Increased frequency of HLA-DR2 in patients with paroxysmal nocturnal hemoglobinuria and the PNH/aplastic anemia syndrome. *Blood*. 2001;98:3513-3519.
11. Bouma G, Crusius JB, Oudkerk PM, et al. Secretion of tumour necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in the TNF genes and HLA-DR alleles: relevance for inflammatory bowel disease. *Scand J Immunol*. 1996;43: 456-463.
12. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-alpha promoter polymorphism effects transcription. *Mol Immunol*. 1997;34:391-399.
13. Sharma S, Ghosh B, Sharma SK. Association of TNF polymorphisms with sarcoidosis, its prognosis and tumour necrosis factor (TNF)-alpha levels in Asian Indians (review). *Clin Exp Immunol*. 2008;151:251-259.
14. Hirankarn N, Avihingsanon Y, Wongpiyabovorn J. Genetic susceptibility to SLE is associated with TNF-alpha gene polymorphism -863, but not -308 and -238, in Thai population. *Int J Immunogenet*. 2007;34:425-430.
15. Skoog T, Hamsten A, Eriksson P. Allele-specific chromatin remodeling of the tumor necrosis factor-alpha promoter. *Biochem Biophys Res Commun*. 2006;351:777-783.
16. Udalova IA, Richardson A, Denys A, et al. Functional consequences of a polymorphism affecting NF-kappaB p50-p50 binding to the TNF promoter region. *Mol Cell Biol*. 2000;20:9113-9119.
17. Deeg HJ, Storer B, Slattery JT, et al. Conditioning with targeted busulfan and cyclophosphamide for hemopoietic stem cell transplantation from related and unrelated donors in patients with myelodysplastic syndrome. *Blood*. 2002;100:1201-1207.
18. Radich JP, Gooley T, Bensinger W, et al. HLA-matched related hematopoietic cell transplantation for chronic-phase CML

using a targeted busulfan and cyclophosphamide preparative regimen. *Blood*. 2003;102:31-35.
19. Anderson JE, Appelbaum FR, Schoch G, et al. Relapse after allogeneic bone marrow transplantation for refractory anemia is increased by shielding lungs and liver during total body irradiation. *Biol Blood Marrow Transplant*. 2001;7:163-170.
20. Flowers MED, Vogelsang GB. Clinical manifestations and natural history. In: Vogelsang GB, Pavletic SZ, editors. *Chronic Graft Versus Host Disease: Interdisciplinary Management*. New York: Cambridge University Press; 2009. p. 56-69.
21. Mielcarek M, Martin PJ, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood*. 2003;102:756-762.
22. Mielcarek M, Storer BE, Flowers MED, et al. Outcomes among patients with recurrent high-risk hematologic malignancies after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2007;13:1160-1168.
23. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. *Blood*. 1998;92:3515-3520.
24. Lin M-T, Storer B, Martin PJ, et al. Relation of an interleukin-10 promoter polymorphism to graft-versus-host disease and survival after hematopoietic-cell transplantation. *N Engl J Med*. 2003;349:2201-2210.
25. Fisher LD, van Belle G. *Biostatistics: A Methodology for the Health Sciences*. New York: Wiley; 1993.
26. Middleton PG, Taylor PRA, Jackson G, et al. Cytokine gene polymorphisms associating with severe acute graft-versus-host disease in HLA-identical sibling transplants. *Blood*. 1998;92:3943-3948.
27. Takahashi H, Furukawa T, Hashimoto S, et al. Contribution of TNF-alpha and IL-10 gene polymorphisms to graft-versus-host disease following allo-hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2000;26:1317-1323.
28. Bertinetto FE, Dall'Omo AM, Mazzola GA, et al. Role of non-HLA genetic polymorphisms in graft-versus-host disease after haematopoietic stem cell transplantation. *Int J Immunogenet*. 2006;33:375-384.
29. Shaw BE, Maldonado H, Madrigal JA, et al. Polymorphisms in the TNFA gene promoter region show evidence of strong linkage disequilibrium with HLA and are associated with delayed neutrophil engraftment in unrelated donor hematopoietic stem cell transplantation. *Tissue Antigens*. 2004;63:401-411.
30. Gersuk GM, Beckham C, Loken MR, et al. A role for tumor necrosis factor-a, Fas and Fas-ligand in marrow failure associated with myelodysplastic syndrome. *Br J Haematol*. 1998;103:176-188.
31. Bornhauser M, Storer B, Slattery JT, et al. Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. *Blood*. 2003;102:820-826.
32. Socié G, Clift RA, Blaise D, et al. Busulfan plus cyclophosphamide compared with total-body irradiation plus cyclophosphamide before marrow transplantation for myeloid leukemia: long-term follow-up of the 4 randomized studies. *Blood*. 2001;98:3569-3574.
33. Slattery JT, Clift RA, Buckner CD, et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. *Blood*. 1997;89:3055-3060.
34. Deeg HJ, Storer BE, Boeckh M, et al. Reduced incidence of acute and chronic graft-versus-host disease with the addition of thymoglobulin to a targeted busulfan/cyclophosphamide regimen. *Biol Blood Marrow Transplant*. 2006;12:573-584.
35. Sandmaier BM, Storb R. Nonmyeloablative therapy and hematopoietic cell transplantation for hematologic disorders. In: Blume KG, Forman SJ, Appelbaum FR, editors. *Thomas' Hematopoietic Cell Transplantation*. Oxford, UK: Blackwell; 2004. p. 1164-1176.