ASBMT American Society for Blood and Marrow Transplantation

Immunosuppressive Cytokine Gene Polymorphisms and Outcome after Related and Unrelated Hematopoietic Cell Transplantation in a Chinese Population

Haowen Xiao,[1,2] Weijie Cao,[1] Xiaoyu Lai,[1] Yi Luo,[1] Jimin Shi,[1] Yamin Tan,[1] Jingsong He,[1] Wanzhuo Xie,[1] Xiaojian Meng,[1] Weiyan Zheng,[1] Gaofeng Zheng,[1] Xiaoyan Han,[1] Lai Jin,[1] Lifei Zhang,[1] Yingjia Wang,[1] Xiaohong Yu,[1] Zhen Cai,[1] Maofang Lin,[1] Xiujin Ye,[1] He Huang[1]

Cytokine gene polymorphisms can affect the outcome of allogeneic hematopoietic stem cell transplantation. We analyzed 6 single nucleotide polymorphisms in 3 immunosuppressive cytokine genes, TGFβ1−509(C>T), +869(T>C), TGFβ1 receptor II (TGFβ1RII) +1167(C>T, codon389 AAC/AAT), and IL-10−1082(A>G), −819(T>C), −592(A>C), in a cohort of 138 pairs of recipients and their unrelated donors and a second cohort of 102 pairs of recipients and their HLA-identical sibling donors. TGFβ1−509 T/T genotype in the donors or T allele-positivity in the recipients was associated with a significant protective effect against acute graft-versus-host disease (aGVHD) and grades II–IV aGVHD in the unrelated transplantation cohort. In the combined cohort, multivariate analysis confirmed that donors with the TGFβ1-509 T/T genotype also conferred protection against the risk of aGVHD and grades II–IV aGVHD. In both the unrelated transplantation cohort and the sibling transplantation cohort, the IL-10−819 C/C and −592 C/C genotypes in either recipients or donors were significantly associated with a higher incidence of aGVHD. In the combined cohort, the IL-10 promoter haplotype polymorphisms at positions −1082, −819, and −592 influenced the occurrence of aGVHD and death in remission. Recipients without the A-T-A haplotype or those transplanted from donors without the A-T-A haplotype had a higher incidence of aGVHD than those who were A-T-A homozygotes or heterozygotes. Estimates for death in remission showed a clear advantage for recipients transplanted from donors with the A-T-A haplotype. In multivariate analysis, recipients without the A-T-A IL-10 haplotype had a higher risk of aGVHD (relative risk [RR] = 0.764; 95% confidence interval [CI]: 0.460-1.269; $P = .096$) and grades II–IV aGVHD (RR = 0.413; 95% CI: 0.245-0.697; $P = .001$). These results provide the first report of an association between TGFβ1, TGFβ1RII, and IL-10 polymorphic features and outcome of allo-HSCT in a Chinese population, and suggest an interaction between TGFβ1−509 genotypes and IL-10 promoter haplotype polymorphisms at positions −1082, −819, and −592 and the risk of aGVHD.

Biol Blood Marrow Transplant 17: 542-549 (2011) © *2011 American Society for Blood and Marrow Transplantation*

KEY WORDS: Hematopoietic stem cell transplantation, TGFβ1, TGFβ1RII, IL10, Polymorphism, GVHD

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective treatment for hematopoietic malignancies, as well as for immune deficiencies and metabolic disorders. In patients undergoing allo-HSCT, the toxicity of the conditioning regimen, infectious complications, and the alloimmune response mediated by donor lymphocytes are all associated with the generation of cytokines. Acute graft-versus-host disease (aGVHD) remains a significant cause of treatment-related mortality and morbidity following allo-HSCT. The incidence of aGVHD ranges from 35% to 45% in recipients after HLA fully matched sibling transplantation, to 60% to 80% in recipients after

From the [1]Bone Marrow Transplantation Center, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang province, People's Republic of China; and [2]Department of Haematology, Guangzhou Liuhuaqiao Hospital, Guangzhou, Guangdong province, People's Republic of China.

Financial disclosure: See Acknowledgments on page 549.

Correspondence and reprint requests: He Huang, MD, PhD, Bone Marrow Transplantation Center, The First Affiliated Hospital, Zhejiang University School of Medicine, No. 79 Qingchun Rd., Hangzhou, 310003, Zhejiang Province, P.R. China (e-mail: hehuang.zju@gmail.com).

Received January 9, 2010; accepted April 20, 2010

1083-8791/$36.00
doi:10.1016/j.bbmt.2010.04.013

1-antigen HLA mismatched, unrelated donor (URD) transplantation [1]. aGVHD can be fatal in 15% to 40% of cases [2]. The imbalance between Th1 cytokines such as tumor necrosis factor (TNF)α, interferon (IFN)γ, and interleukin (IL)1, and Th2 cytokines such as IL-4, IL-10, and the immunoregulatory cytokine, transforming growth factor-β (TGFβ), has been suggested to play an important role in the development of aGVHD.

TGFβ and IL-10 are pleiotropic regulatory cytokines in the immune system, and both play key roles in the function of regulatory T cells (Treg). Furthermore, IL-10 facilitates the regulatory function of TGFβ [3-5]. Clinical data and data from animal models suggest that TGFβ and IL-10 can suppress aGVHD [6,7]. The potential to generate cytokines may be associated with polymorphic features of the cytokine-encoding genes. Many cytokine gene polymorphisms, such as those for TNFα, IL-10, and IFNγ, have been investigated over the last 10 years for their potential roles in the occurrence and severity of GVHD, as well as for their contribution to overall treatment-related mortality, infectious episodes, and overall survival (OS) [8,9]. However, very few studies have simultaneously studied sibling donor and URD transplantation, and there are no data in the Chinese population. The present study was designed to test the influence of polymorphisms of the immunosuppressive cytokine genes for TGFβ and IL-10 on the outcome of allo-HSCT in a cohort of 138 pairs of recipients and their URDs, and in a second cohort of 102 pairs of recipients and their HLA-identical sibling donors.

MATERIALS AND METHODS

Characteristics of the HSCT Patient Group

The entire study population consisted of 240 pairs of transplant recipients and their donors who were transplanted from January 2001 to March 2009 in our Bone Marrow Transplantation Unit. The incidences of aGVHD, chronic GVHD (cGVHD) and OS were analyzed in relation to IL-10, TGFβ1, and TGFβ1 receptor II (TGFβ1RII) gene polymorphisms (Table 1). All the patients and their donors were of Chinese origin. The study was approved by the local ethics committee. All the patients and donors gave their written informed consent.

Low-resolution HLA typing had been performed for HLA-A, -B, and -DRB1 in sibling transplantation and high-resolution DNA typing for HLA-A, -B, -C, -DRB1, and -DQB1 in URD transplantation.

The main myeloablative (MA) conditioning regimens used were busulfan/cyclophosphamide (BuCy) without total body irradiation (TBI); reduced-intensity conditioning regimens (RIC) were predominantly fludarabine-based combinations without irradiation. Both in the unrelated and sibling transplantation cohorts, the patients received the same GVHD prophylaxis consisting of cyclosporine A, a short-term methotrexate (MTX), and mycophenolate mofetil (MMF).

The study was divided into 2 phases and involved 2 separate cohorts. The initial cohort consisted of 138 pairs of recipients and their URD. We used this cohort to screen for an association between GVHD and IL-10, TGFβ1, and TGFβ1RII gene polymorphisms. The second cohort included 102 pairs of recipients and their HLA-identical sibling donors. This cohort was used for confirmatory analysis. There were significant differences between these groups in terms of the patients' ages, transplant material, cumulative incidence of aGVHD, cumulative incidence of death in remission, and OS (Table 1). A final analysis of clinical end points included both cohorts.

DNA Extraction

Genomic DNA was extracted from peripheral blood samples obtained from recipients and donors before transplantation using a salting-out method with a commercial DNA extraction kit (Dynal Biotech, Brown Deer, WI), following the manufacturer's recommendations. DNA was quantified by spectrophotometry.

Analysis of TGFβ1, TGFβ1RII, and IL10 Polymorphisms

The IL-10-1082(A>G), −819(T>C), −592 (A>C), TGFβ1−509(C>T), +869 (T>C), and TGFβ1RII+1167(C>T, codon389 AAC/AAT) single nucleotide polymorphisms (SNPs) were determined by multiplex SNaPshot technology (according to previously described methods [10-12]), using an ABI fluorescence-based assay allelic discrimination method (Applied Biosystems, Bedford, MA).

The primers for polymerase chain reaction (PCR) amplification and SNaPshot extension reactions were both designed to be aligned with the NCBI sequence databases using Primer3 software. The extension primer was designed to anneal immediately adjacent to the nucleotide at the mutation site, on either the sense or antisense DNA strand (Table 2).

PCR was carried out in a total volume of 10 μL containing 50 ng genomic DNA, 0.1 μM of each primer, 0.3 mM each of dATP, dCTP, dTTP, and dGTP, 1 unit of HotStarTaq polymerase (Qiagen, Chatsworth, CA), 4 μL of 1× buffer, and 3.0 mM $MgCl_2$. The samples were put through 30 to 40 cycles of denaturation at 94°C, annealing at specific primer temperatures, elongation at 72°C, and a final extension at 72°C. PCR product amplification was verified by running 5 μL of product on a 2% agarose gel. The

Table 1. Patients, Disease, and Transplantation Characteristics in the First and Second Cohorts

Characteristics	Unrelated Transplant (n = 138)	Sibling Transplant (n = 102)	*P*-Value
Age (median, range), years	24 (10∼50)	32 (14-52)	<.001
Donor-recipient sex, n (%)			
Female-male	35 (25.4)	33 (32.4)	.249
Other	103 (74.6)	69 (67.6)	
Reason for transplantation, n (%)*			
Nonmalignant disease	6 (4.3)	6 (5.9)	.766
Low-risk cancer	117 (84.8)	90 (88.2)	.57
High-risk cancer	15 (10.9)	6 (5.9)	.248
Transplant material, n (%)			
Bone marrow	56 (40.6)	12 (12.8)	<.001
Peripheral blood stem cells	82 (59.4)	90 (87.2)	
HLA matching			
Matched	96 (69.6)	102 (100%)	<.001
Mismatched	42 (30.4)	0	
Conditioning regimen, n (%)			
Myeloablative	120 (87)	89 (87.3)	.946
RIC	18 (13.0)	13 (12.7)	
Cumulative incidence of aGVHD	%	%	
Grade 0	31.9	76.5	<.001
Grade I	24.6	10.8	.007
Grade II	32.6	11.8	.003
Grade III	2.2	1.0	<.001
Grade IV	8.7	0	<.001
Cumulative incidence of cGVHD, (%)	32.6	33.3	.933
Cumulative incidence of clinical extensive cGVHD, (%)	15.9	10.8	.343
Cumulative incidence of relapse, (%)	21.0	19.6	.872
Cumulative incidence of death in remission, (%)	24.6	9.8	.004
Cumulative overall survival, %	58	74.5	.009

RIC indicates reduced-intensity conditioning; cGVHD, chronic graft-versus-host disease; aGVHD, acute graft-versus-host disease.
*Nonmalignant diseases included aplastic anemia, myelodysplastic syndrome, and paroxysmal nocturnal hematuria. Low-risk cancers included acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), and non-Hodgkin lymphoma (NHL) in complete remission (CR) and chronic myelogenous leukemia (CML) in chronic phase. High-risk cancers included ALL, AML, and NHL in relapse; CML in other than chronic phase; multiple myeloma, and Hodgkin's disease.

remaining product was then processed according to the ABI SNaPshot protocol, using primers designed for fluorescence dideoxynucleotide termination. SNP analysis was carried out using an ABI3130 genetic analyzer. Genotypes were determined automatically using Genemapper4.0 software (Applied Biosystems). Genotyping was confirmed by sequencing in 10% of randomly selected samples, as a quality control measure.

Statistical Analyses

Only patients who had successful engraftment and survived more than 30 days were included in the analysis of aGVHD. Univariate analyses of the distribution of IL-10, TGFβ1, and TGFβ1RII genotypes in patients with and without aGVHD and cGVHD were performed using Fisher's exact tests. The incidences of relapse, death in remission, and survival were estimated using the Kaplan-Meier method, and the generalized Wilcoxon test was used to analyze the differences. The Cox proportional hazard model was applied to multivariate analysis of the effects of these characteristics on HSCT outcome. Statistical analysis was performed using SPSS software version 16.0. All probability values were 2 sided. A value of $P < .05$ was considered to be statistically significant, and values of P between .05 and .1 were considered to be indicative of a trend.

RESULTS

Polymorphisms of TGFβ1, TGFβ1RII, and IL-10 in the First and Second Cohorts

In our study, the frequencies of the TGFβ1, TGFβ1RII, and IL-10 genotypes in recipients and donors were almost equal, and were consistent with previously reported results for the Chinese population and with the NCBI SNP databases. There were also no significant differences in the distribution of TGFβ1, TGFβ1RII, and IL-10 genotypes in the first URD transplantation cohort and second sibling transplantation cohort (Table 3). No significant deviations from the Hardy-Weinberg expected frequencies of these genotypes were observed in either donors or recipients (data not shown).

Association of TGFβ1, TGFβ1RII, and IL-10 Gene Polymorphisms with aGVHD in the First Cohort

In the first cohort consisting of 138 pairs of recipients and their unrelated donors (Table 4), the TGFβ1−509 T allele in either recipients or donors was significantly associated with a lower incidence of

Table 2. Primers Sequences Used in the SNaPshot Reaction

Mutation Position	PCR Amplification Primers	Extension Primers
for IL10		
−1082 A>G (rs1800896)	F TCCCCAGGTAGAGCAACACTC	F TTTTTTTTTTTTTTTTTTTTTAAC
	R ATGGAGGCTGGATAGGAGGTC	ACTACTAAGGCTTCTTTGGGA
−819 T>C (rs1800871)	F GGTGAGGAAACCAAATTCTCA	F TTTTTTTTTTTTTTTTTTGTGTA
	R CAAGCAGCCCTTCCATTTTAC	CCCTTGTACAGGTGATGTAA
−592 A>C (rs1800872)		R TTTTTTTTTTTTTTTTTTTTTTT
		TTTTTTCCAGAGACTGGCTTCC TACAG
for TGFβ1		
−509 C>T (rs1800469)	F GCAGGGTGTTGAGTGACAGG	R TTTTGGGCAACAGGACACCTA
	R GAGGGTGTCAGTGGGAGGAG	
+869 T>C (rs1800470)	F CTACCTTTTGCCGGGAGACC	F TTTTTTTTCCGGGCTGCGGCTGC TGC
	R GTCAGCACCAGTAGCCACAGC	
for TGFβ1RII		
+1167 C>T (rs2228048)	F GATTGCTCACCTCCACAGTGA	R TTTTTTTTTTCACAGGCAGCAG
	R CCACTGTTAGCCAGGTCATCC	GTTAGGTC

aGVHD and grades II–IV aGVHD. The incidences of aGVHD and grades II–IV aGVHD in T allele-positive recipients were 63.1% and 36.9%, respectively, in contrast to 82.9% and 62.9%, respectively, in C/C genotype recipients ($P = .036$, $P = .01$, respectively). In recipients transplanted from T/T genotype donors, the incidences of aGVHD and grades II–IV aGVHD were 52.9% and 20.6%, respectively, in contrast to 73.1% and 51.0%, respectively, with other genotypes ($P = .035$, $P = .003$, respectively).

When the association of IL-10 genotypes with aGVHD was examined, donors or recipients with IL-10–819 C/C genotype were associated with a higher incidence of aGVHD in recipients compared with T allele-positive donors or recipients (donor side: 85.7% versus 63.6%, $P = .025$; recipient side: 87.5% versus 64%, $P = .03$, respectively). A similar association was observed for the IL-10−592 C/C genotype in recipients or donors compared with A allele-positivity ($P < .05$). Furthermore, the IL-10–819 C/C or −592 C/C genotypes in either recipients or donors were both associated with a higher incidence of grades II–IV aGVHD (IL10−819 C/C genotype in the donor side $P = .054$, in the recipient side $P < .001$; IL10−592 C/C genotype in the donor side $P = .084$, in the recipient side $P = .001$). No significant associations between TGFβ1+869, TGFβ1RII+1167, or IL-10−1082 genotypes in either recipients or donors and aGVHD were detected.

HLA allele mismatching is the main cause of aGVHD development. To distinguish between the

Table 3. Distribution of TGFβ1, TGFβ1RII, and IL10 Genotypes in the Donors and Recipients in the Unrelated and Sibling Transplantation Cohorts

	Donor Type			Recipient Type		
Genotype	Unrelated Cohort n (%)	Sibling Cohort n (%)	*P*-Value	Unrelated Cohort n (%)	Sibling Cohort n (%)	*P*-Value
TGFβ1−509			>.05			>.1
C/C	32 (23.2)	34 (33.3)		35 (25.4)	36 (35.3)	
T/C	72 (52.2)	53 (52)		66 (47.8)	47 (46.1)	
T/T	34 (24.6)	15 (14.7)		37 (26.8)	19 (18.6)	
TGFβ1+869			>.05			>.05
T/T	30 (21.7)	31 (30.4)		31 (22.5)	33 (32.4)	
C/T	71 (51.4)	54 (52.9)		68 (49.2)	51 (50)	
C/C	37 (26.8)	17 (16.7)		39 (28.3)	18 (17.6)	
TGFβ1RII +1167			>.1			>.1
C/C	69 (50.0)	57 (55.9)		76 (55.1)	55 (53.9)	
T/C	52 (37.7)	37 (36.3)		49 (35.5)	38 (37.3)	
T/T	17 (12.3)	8 (7.8)		13 (9.4)	9 (8.8)	
IL10-1082			>.1			>.1
A/A	121 (87.7)	91 (89.2)		117 (84.8)	90 (88.2)	
G/A	14 (10.1)	11 (10.8)		18 (13.0)	11 (10.8)	
G/G	3 (2.2)	0 (0)		3 (2.2)	1 (1.0)	
IL10−819			>.1			>.1
T/T	60 (43.5)	48 (47.1)		56 (40.6)	39 (38.2)	
T/C	50 (36.2)	39 (38.2)		58 (42.0)	45 (44.1)	
C/C	28 (20.3)	15 (14.7)		24 (17.4)	18 (17.6)	
IL10-592			>.1			>.1
A/A	57 (41.3)	48 (47.1)		56 (40.6)	45 (44.1)	
A/C	54 (39.1)	39 (38.2)		60 (43.5)	40 (39.2)	
C/C	27 (19.6)	15 (14.7)		22 (15.9)	17 (16.7)	

The differences of the distribution of TGFβ1, TGFβ1RII, and IL-10 genotypes in the first unrelated transplantation cohort and second sibling transplantation cohort were performed using Fisher's exact tests.

Table 4. Association of Cytokine Gene Polymorphisms and aGVHD in the First Cohort of Total and HLA-Matched Recipients and Unrelated Donors

	Donor Type				Recipient Type			
Genotype	Incidence of aGVHD(%)	*P*-Value	Incidence of II-IV aGVHD(%)	*P*-Value	Incidence of aGVHD(%)	*P*-Value	Incidence of II-IV aGVHD(%)	*P*-Value
Total cohort (n = 138)								
TGFβ1−509								
T/T	52.9	**.035**	20.6	**.003**	T+ 63.1	**.036**	36.9	**.01**
T/C+C/C	73.1		51.0		CC 82.9		62.9	
TGFβ1+869								
C/C	56.8	.1	35.1	.251	65.0	.688	37.5	.45
T/C+T/T	72.3		46.5		69.4		45.9	
TGFβ1RII +1167								
C/C	75.0	.143	40.5	.494	63.2	.268	40.3	.489
T/C+T/T	62.2		46.9		73.3		47.5	
IL10-1082								
A/A	69.4	.411	43.8	1.0	65.8	.210	41.5	.331
G/A+G/G	58.8		41.2		81.0		55.0	
IL10-819								
C/C	85.7	.025	60.7	**.054**	87.5	**.03**	79.2	**<.001**
C/T+T/T	63.6		39.1		64.0		36.0	
IL10-592								
C/C	85.2	**.039**	59.3	**.084**	86.4	**.049**	77.3	**.001**
C/A+A/A	64.0		39.6		64.7		37.1	
HLA-matched (n = 96)								
TGFβ1-509								
T/T	51.9	.154	18.5	.019	T+ 59.7	.138	31.9	.087
T/C+C/C	69.6		44.9		CC 79.2		54.2	
IL10-819								
C/C	87.5	**.045**	56.2	**.09**	80.0	.243	66.7	**.018**
C/T+T/T	60.0		33.8		61.7		32.1	
IL10-592								
C/C	86.7	**.076**	53.3	.245	78.6	.366	64.3	**.036**
C/A+A/A	60.5		34.6		62.2		32.9	

aGVHD indicates acute graft-versus-host disease.
Univariate analyses of aGVHD and cytokine genotype by Fisher's exact tests. *P*-values <.1 are given in bold font.

effects of HLA mismatching and those of cytokine gene polymorphisms, high-resolution HLA-A, -B, -C, -DRB1, and -DQB1 allele-matched cases (n = 96) were analyzed (Table 4). In this analysis, we found similar trends. TGFβ1−509 T/T genotype in the donors or T allele-positivity in the recipients was also associated with a lower incidence of grades II–IV aGVHD (donor side: $P = .019$; recipient side: $P = .087$). The IL-10−819 C/C or −592 C/C genotypes in donors were associated with a higher incidence of aGVHD and in recipients with a higher incidence of grades II–IV aGVHD.

Association of TGFβ1, TGFβ1RII, and IL-10 Gene Polymorphisms with aGVHD in the Second Cohort

To distinguish between the effects of the combined influence of other genetic disparities and those of cytokine gene polymorphisms, the TGFβ1−509, IL-10−819, and IL-10−592 polymorphisms that showed a significant association with the incidence of aGVHD in the unrelated transplantation cohort were confirmed in a second cohort consisting of 102 pairs of recipients and their HLA-identical sibling donors (Table 5). The TGFβ1−509 T/T genotype showed a protective effect against aGVHD, although the association was not significant. The IL-10−819 C/C in either recipients or donors and IL-10−592 C/C genotypes in donors were both significantly associated with a higher incidence of aGVHD. Although the small number of recipients with severe aGVHD precluded statistical analysis of the relationship between cytokine gene polymorphisms and severe aGVHD, the agreement between the 2 independent cohorts suggested that TGFβ1−509, IL-10−819, and IL-10−592 polymorphisms in the recipients or donors were associated with the risk of aGVHD.

Polymorphisms of TGFβ1, TGFβ1RII, and IL-10 Promoter Region Haplotype and Transplantation Outcome

Neither among the first unrelated transplantation cohort nor second sibling transplantation cohort was there association between donor or recipient TGFβ1 and TGFβ1RII genotype and incidence of cGVHD or extensive cGVHD. Kaplan-Meier analysis found no significant associations between donor or recipient TGFβ1 and TGFβ1RII genotypes and relapse rate, incidence of death in remission, or OS (data not shown).

The results from the 2 independent cohorts both suggested that the IL-10−819 C/C and IL-10−592 C/C genotypes in either recipients or donors were significantly associated with higher incidences of aGVHD. Previous studies have identified linkage disequilibrium between the IL-10−819 SNP (T or C allele) and the −592 SNP (A or C allele), with only 2 haplotypes observed at reasonable frequencies. In 1, −819T is linked to −592A (the T-A haplotype), and in the other, −819C is linked to −592C (the C-C haplotype) [13]. In the combined cohort of 240 pairs of donors and recipients, we stratified donors and recipients according to promoter haplotypes of IL-10 (A-T-A, A-C-C, and G-C-C), defined by SNPs at positions −1082, −819, and −592, to examine the relative effects of the individual haplotype on the risk of aGVHD. Among the resulting genotypes, the incidence of aGVHD was higher in the group without the A-T-A haplotype than in the group with the A-T-A haplotype, either in donors or recipients (donor side: 71.4% versus 44.4%, $P = .002$; recipient side: 67.5% versus 45.5, $P = .015$). The same tendency was found for the incidence of grades II–IV aGVHD (donor side: 47.6% versus 26.8%, $P = .01$; recipient side: 57.5% versus 25.0, $P < .001$). However, the A-T-A haplotype showed no significant protective effect against the occurrence of cGVHD or extensive cGVHD.

Estimates for death in remission showed a clear advantage for recipients transplanted from donors with the A-T-A haplotype (Figure 1A; $P = .05$). Recipients with the A-T-A haplotype also showed a lower incidence of death in remission, although the difference was not significant (Figure 1B; $P = .195$). No significant associations were found between donor or recipient IL-10 promoter haplotypes and relapse rate or OS.

Multivariate Analysis of Risk Factors Associated with aGVHD

TGFβ1−509 polymorphisms and IL-10 haplotype, together with other clinical and biological factors known to contribute to the development of aGVHD, were subjected to multivariate Cox proportional hazard model analysis for aGVHD. The following factors were analyzed: sibling donor versus URD, transplant material (peripheral blood stem cells versus bone marrow), donor-recipient sex relation (male recipient with female donor, or other), HLA matching, conditioning regimen (MA conditioning versus RIC).

The results are given in Table 6. Three variables were significantly associated with the risk of aGVHD. MA conditioning (relative risk [RR] = 3.423, $P = .002$), donor female and recipient male (RR = 1.595, $P = .017$), and URD (RR = 4.467, $P < .001$) were found to significantly contribute to the development of aGVHD. A donor with TGFβ1−509 C allele-positivity (RR = 1.436, $P = .074$) and a recipient without the A-T-A IL-10 haplotype (RR = 0.764, $P = .096$) were found to be less significant factors influencing the risk of aGVHD. MA conditioning, an URD, a donor with TGFβ1−509 C allele-positivity, and a recipient without the A-T-A IL-10 haplotype also all contributed significantly to the development of grades II–IV aGVHD ($P < .05$). A donor female and

Table 5. Association of Cytokine Gene Polymorphisms and aGVHD in the Second Cohort of 102 Pairs of Recipients and Sibling Donors

	Donor Type		Recipient Type	
Genotype	Incidence of aGVHD(%)	*P*-Value	Incidence of aGVHD(%)	*P*-Value
TGFβ1−509				
T/T	7.1	.178	10.5	.229
T/C+C/C	26.1		26.5	
IL10−819				
C/C	46.7	**.042**	44.4	**.032**
C/T+T/T	19.5		19.0	
IL10−592				
C/C	46.7	**.042**	41.2	.113
C/A+A/A	19.5		20.0	

aGVHD indicates acute graft-versus-host disease.
Univariate analyses of aGVHD and cytokine genotype by Fisher's exact tests. *P*-values <.1 are given in bold font.

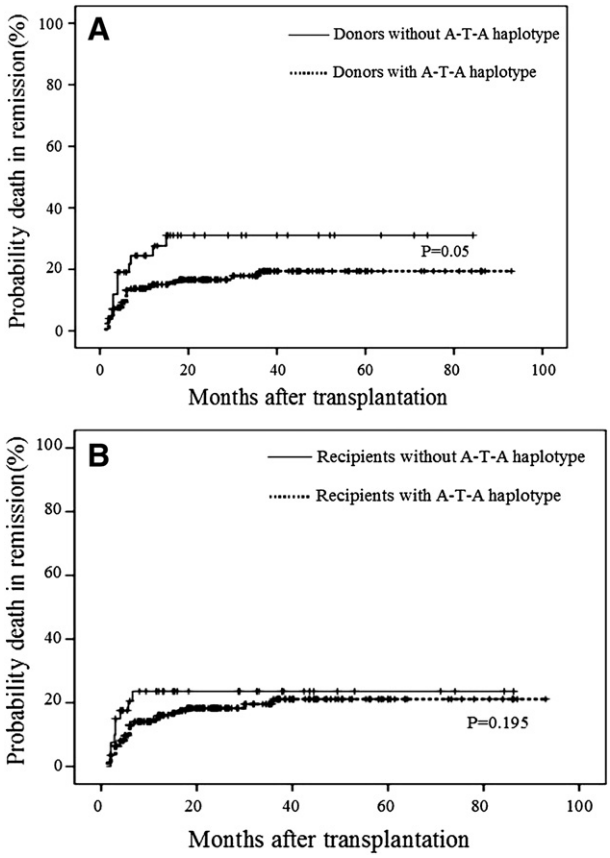

Figure 1. Cumulative incidence of death in remission according to the promoter haplotypes of IL-10 among transplantation donors (A) and recipients (B) by Kaplan-Meier analysis.

Table 6. Multivariate Analysis of Risk Factors for aGVHD and Grades II-IV aGVHD

Variable	aGVHD		Grades II-IV aGVHD	
	RR (95%CI)	*P*	RR (95%CI)	*P*
Myeloablative conditioning	3.423 (1.592-7.360)	.002	4.764 (1.490-15.227)	.008
Donor female and recipient male	1.595 (1.085-2.343)	.017	1.456 (0.871-2.434)	.098
Unrelated donor	4.467 (2.837-7.032)	<.001	6.652 (3.538-12.509)	.001
Donor with TGFβ1−509 C allele-positivity	1.436 (0.852-2.421)	.074	2.879 (1.301-6.374)	.009
Recipient without A-T-A haplotype of IL10	0.764 (0.460-1.269)	.096	0.413 (0.245-0.697)	.001

RR indicates relative risk; aGVHD, acute graft-versus-host disease.

a recipient male was found to be a less significant factor influencing the risk of grades II–IV aGVHD (RR = 1.456, $P = .098$).

DISCUSSION

Analyses of the outcome of the 2 separate cohorts in the present study demonstrated a higher incidence of aGVHD, severe aGVHD, and death in remission in the URD transplantation cohort. OS was also higher in the sibling transplantation cohort. We were able to corroborate previous findings suggesting that unrelated donor, MA conditioning, and a donor female and a recipient male are risk factors for aGVHD. The reduced incidence of aGVHD following RIC compared to MA conditioning may be because of less tissue damage and lower levels of inflammatory cytokines [14,15]. The higher incidence of aGVHD in the URD and a female donor-to-male recipient transplantation may be because of HLA and non-HLA genetic divergence, such as sex-related Y chromosome-encoded minor histocompatibility antigens and cytokine gene polymorphisms [16-18]. Thus, HLA and non-HLA genetics and cytokines are important factors in the pathogenesis of aGVHD.

TGFβ includes TGFβ1, TGFβ2, and TGFβ3. The major form expressed in the immune system and present in the blood is TGFβ1. TGFβ1 controls T cell tolerance via direct inhibition of Th1, Th2, and cytotoxic T lymphocyte differentiation and the maintenance of Treg cells [3]. A previous study showed that the concentration of TGFβ1 in plasma was unaffected by environmental factors, such as age, body mass index, or drugs taken [19]. However, the concentration of TGFβ1 was genetically regulated. A study of 170 pairs of female twins found that the presence of the T allele at TGFβ1−509 was associated with higher concentrations of TGFβ1, and this increase in concentration was greater among T/T homozygotes than T heterozygotes. The mean TGFβ1 concentration was approximately twice as high in T/T compared with C/C homozygotes [20]. We found that donor TGFβ1−509 T/T genotype or recipient T allele-positivity were associated with significant protective effects against aGVHD and grades II–IV aGVHD in the unrelated transplantation cohort. Multivariate analysis confirmed that donors with the TGFβ1−509 T/T genotype reduced the risk of aGVHD and grades II-IV aGVHD, possibly because of higher production of the immunosuppressive cytokine, TGFβ1. A Japanese study of 67 pairs of children recipients and their HLA-identical sibling donors found that TGFβ1+869 and TGFβ1RII+1167 polymorphisms were associated with the development of aGVHD, whereas TGFβ1−509 genotype had no significant influence [21]. A study in an Iranian population also showed the association between TGFβ1+869 polymorphism and aGVHD [22]. The discrepancies between the results of different studies could be because of heterogeneity among the patients, donor type, conditioning regimens, GVHD prophylaxis, or incidence of aGVHD.

IL-10 is a well-known suppressive cytokine of T cell proliferation and cytokine production, and plays a pivotal role in the establishment of peripheral T cell tolerance. Previous studies have observed an association between elevated levels of IL-10 and reduced risk of aGVHD [23,24]. In both the unrelated and sibling transplantation cohorts we found that the IL-10–819 C/C and −592 C/C genotypes in either recipients or donors were significantly associated with a higher incidence of aGVHD. In the combined cohort, the IL-10 promoter haplotype polymorphisms at positions −1082, −819, and −592 affected the occurrence of aGVHD and death in remission. Recipients without the A-T-A haplotype or those transplanted from donors without the A-T-A haplotype had a higher incidence of aGVHD and death in remission than A-T-A homozygotes or heterozygotes. This specific effect could be because of increased IL-10 production in individuals with the A-T-A haplotype, as previously reported [13,25,26]. A study of 570 pairs of transplant recipients and their HLA-identical sibling donors also demonstrated that the A-T-A haplotype was associated with a lower risk of aGVHD [13], although the same study group failed to observe the samilar effects in a cohort of patients and their unrelated donors [27].

In the current study, no significant associations were found between cytokine gene polymorphisms and cGVHD, relapse, or OS. This could be because of differences in the pathophysiology of aGVHD and cGVHD and stronger immunosuppressive therapy in

patients with aGVHD, which causes a reduction in the correlation between cytokine gene polymorphisms and cGVHD. Numerous influences could affect the relapse and OS rates and reduce the influence of cytokine gene polymorphisms.

The results of previous studies of non-HLA immunogenetics, including cytokine gene polymorphisms, in HSCT have been inconsistent. Specialists have concentrated on the influences of patient heterogeneity, conditioning regimens, and GVHD prophylaxis. However, significant differences in the occurrence of aGVHD between different cohorts, as between our 2 independent cohorts, could also contribute to the inconsistencies. The association may become more apparent if groups at higher risk of GVHD are studied, as in unrelated or mismatched donor transplantation.

This study is first to report the relationship between IL-10, TGFβ1, and TGFβ1RII polymorphisms and the outcome of allo-HSCT within the Chinese population. The results may provide useful information for determining risk assessment and donor selection, and as a guide for selecting appropriate immunosuppressive therapy.

ACKNOWLEDGMENTS

Financial disclosure: This work was funded by Zhejiang Provincial Key Medical Discipline (Medical Tissue Engineering), Major Program of Zhejiang Provincial Science (2006C13022), and Health Foundation of Ministry of Public Health (200802027). We would like to thank Shanghai Genesky Bio-Tech Genetic Core Lab for their excellent technical assistance with genotyping analyses. We also thank for the assistance of HLA Typing Laboratory of Blood Center of Zhejiang Province.

REFERENCES

1. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet*. 2009;373:1550-1561.
2. Sun Y, Tawara I, Toubai T, Reddy P. Pathophysiology of acute graft-versus-host disease: recent advances. *Transl Res*. 2007;150: 197-214.
3. Li MO, Flavell RA. Contextual regulation of inflammation: a duet by transforming growth factor-β and interleukin-10. *Immunity*. 2008;28:468-476.
4. Taylor A, Verhagen J, Blaser K, Akdis M, Akdis CA. Mechanisms of immune suppression by interleukin-10 and transforming growth factor-beta: the role of T regulatory cells. *Immunology*. 2006;117:433-442.
5. Fuss IJ, Boirivant M, Lacy B, Strober W. The interrelated roles of TGF-beta and IL-10 in the regulation of experimental colitis. *J Immunol*. 2002;168:900-908.
6. Ju XP, Xu B, Xiao ZP, et al. Cytokine expression during acute graft-versus-host disease after allogeneic peripheral stem cell transplantation. *Bone Marrow Transplant*. 2005;35:1179-1186.
7. Banovic T, MacDonald KP, Morris ES, et al. TGF-beta in allogeneic stem cell transplantation: friend or foe? *Blood*. 2005;106: 2206-2214.
8. Ball LM, Egeler RM. Acute GVHD: pathogenesis and classification. *Bone Marrow Transplant*. 2008;41:58-64.
9. Dickinson AM. Polymorphisms of cytokine and innate immunity genes and GVHD. *Best Pract Res Clin Haematol*. 2008;21:149-164.
10. Yan H, Yuan W, Velculescu VE, Vogelstein B, Kinzler KW. Allelic variation in human gene expression. *Science*. 2002;297:1143.
11. Donn R, Payne D, Ray D. Glucocorticoid receptor gene polymorphisms and susceptibility to rheumatoid arthritis. *Clin Endocrinol*. 2007;67:342-345.
12. Sistonen J, Fuselli S, Levo A, Sajantila A. CYP2D6 genotyping by multiplex primer extension reaction. *Clin Chem*. 2005;51: 1291-1295.
13. Lin MT, 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.
14. Perez-Simon JA, Diez-Campelo M, Martino R, et al. Influence of the intensity of the conditioning regimen on the characteristics of acute and chronic graft-versus-host disease after allogeneic transplantation. *Br J Haematol*. 2005;130:394-403.
15. 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.
16. Dickinson AM, Harrold JL, Cullup H. Haematopoietic stem cell transplantation: can our genes predict clinical outcome? *Expert Rev Mol Med*. 2007;9:1-19.
17. Petersdorf EW, Hansen JA. New advances in hematopoietic cell transplantation. *Curr Opin Hematol*. 2008;15:549-554.
18. Hambach L, Spierings E, Goulmy E. Risk assessment in haematopoietic stem cell transplantation: minor histocompatibility antigens. *Best Pract Res Clin Haematol*. 2007;20:171-187.
19. Grainger DJ, Kemp PR, Metcalfe JC, et al. The serum concentration of active transforming growth factor-β is severely depressed in advanced atherosclerosis. *Nat Med*. 1995;1:74-79.
20. Grainger DJ, Heathcote K, Chiano M, et al. Genetic control of the circulating concentration of transforming growth factor type β1. *Hum Mol Genet*. 1999;8:93-97.
21. Hattori H, Matsuzak A, Suminoe A, et al. Polyporphisms of transforming growth factor-β1 and transforming growth factor-β1 type II receptor genes are associated with acute grafe-versus-host disease in children with HLA-matched sibling bone marrow transplantation. *Bone Marrow Transplant*. 2002;30:665-671.
22. Noori-Daloii MR, Rashidi-Nezhad A, Izadi P, et al. Transforming growth factor-beta1 codon 10 polymorphism is associated with acute GVHD after allogenic BMT in Iranian population. *Ann Transplant*. 2007;12:5-10.
23. Bacchetta R, Bigler M, Touraine JL, et al. High levels of interleukin10 production in vivo are associated with tolerance in SCID patients with HLA mismatched hematopoietic stem cells. *J Exp Med*. 1994;179:493-502.
24. Holler E, Roncarolo MG, Hintermeier-Knabe R, et al. Prognostic significance of increased IL10 production in patients prior to allogeneic bone marrow transplantation. *Bone Marrow Transplant*. 2000;25:237-241.
25. Keijsers V, Verweij CL, Westendorp RGJ, Breedveld FC, Huizinga TWJ. IL10 polymorphisms in relation to production and rheumatoid arthritis. *Arthritis Rheum*. 1997;40. Suppl:S179.
26. Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL10 promoter affect IL10 production and enhance the risk of systemic lupus erythematosus. *J Immunol*. 2001;166:3915-3922.
27. Tseng LH, Storer B, Petersdorf E, et al. IL10 and IL10 receptor gene variation and outcomes after unrelated and related hematopoietic cell transplantation. *Transplantation*. 2009;87: 704-710.