Published in final edited form as:
Tissue Antigens. 2009 July ; 74(1): 50–56. doi:10.1111/j.1399-0039.2009.01257.x.

TNF, LTA and *TGFB1* genotype distributions among acute graft-*vs*-host disease subsets after HLA-matched unrelated hematopoietic stem cell transplantation: a pilot study

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Abstract

Cytokine single nucleotide polymorphisms and consequent production levels have been associated with acute graft-*vs*-host disease (aGVHD) development. The aim of this pilot study was to determine whether polymorphisms in tumor necrosis factor (*TNF*), lymphotoxin alpha (*LTA*) and transforming growth factor beta 1 (*TGFB1*) showed any association with aGVHD severity. Novel alleles and polymorphisms were identified for each cytokine locus. Genotype distributions were examined in 38 recipient–donor pairs (all chronic myelogenous leukemia in the first chronic phase) with either low-grade (grades 0–I) or high-grade (grades III–IV) aGVHD. Although no significant differences were found, some trends were noted in genotype distributions among aGVHD-grade groups. Power calculations determined that substantially more pairs would be required to show significant associations in distributions among aGVHD-grade groups.

Keywords

cytokines; graft-*vs*-host disease; hematopoietic stem cell transplantation; polymorphism

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for a number of hematological and immune disorders (1, 2). Studies have consistently shown that donor and recipient matching for the major histocompatibility complex (MHC)-encoded human leukocyte antigen (HLA) improves transplantation outcome (3). However, up to 50% of patients develop acute graft-*vs*-host disease (aGVHD) even when HLA-matched allogeneic transplantation is performed in conjunction with rigorous immunosuppressive therapy (4). To prevent and effectively treat aGVHD, it is critical to understand the molecular and cellular events that play a central role in its pathogenesis. For instance, aGVHD, in large part, is a consequence of damage to host tissue by activated donor T cells and cytokines upon recognition of the host as foreign. Thus, it has become increasingly apparent that other immune response genes other than HLA, such as those encoding cytokines, significantly contribute toward transplantation outcome and aGVHD (5, 6).

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Supporting information: Additional Supporting Information may be found in the online version of this article.

Cytokines play an important role in immune modulation. Very high levels of cytokines are observed in patients with aGVHD and the term 'cytokine storm' is often used to describe its pathobiology (7). Various regimens used for transplant conditioning damage host tissues and lead to increased levels of cytokines like tumor necrosis factor (TNF), lymphotoxin alpha (LT-α) and transforming growth factor beta 1 (TGF-β1) in the early phase of aGVHD (8). Subsequent activation and proliferation of donor T cells in the second phase lead to augmentation in cytokine levels resulting in continued activation and recruitment of T cells, macrophages and other effector cell types. Cytokine levels pre- and posttransplant are known to affect transplant outcome (9).

Polymorphisms both in the regulatory and in the coding regions of cytokine genes contribute to interindividual differences in their expression levels and subsequently alter immune responses and affect disease pathogenesis and outcome (10). Indeed, many functional cytokine polymorphisms have been found to affect transplant outcome, development of graft-*vs*-host disease (GVHD) and graft rejection in HSCT (11, 12). However, ambiguities persist in cytokine single nucleotide polymorphism (SNP) and GVHD associations observed among studies (10), which could be caused by a variety of reasons such as study size, race/ethnicity, differences in the degree of HLA mismatch and varying linkages between multiple SNPs that impact expression within a cytokine gene.

In this pilot study, direct DNA sequencing was used to determine known and novel polymorphisms in three important cytokine genes (*TNF*, *LTA* and *TGFB1*), as well as their allelic linkages in 38 HLA allele-matched unrelated HSCT pairs. The distribution of both SNPs and alleles for each cytokine among aGVHD-grade groups (0–I *vs* III–IV) in donors and in recipients was examined to determine associations with severity of aGVHD. To maximize the distinction between the aGVHD-grade groups and the probability of associations with cytokine genotypes, pairs with grade II aGVHD were not included in this study.

The characteristics of the White study participants are shown in Table 1. All transplant pairs were allele matched at HLA-A, -B, -C, -DRB1, -DQA1 and -DQB1. Fifteen of the 38 pairs (39.5%) had one HLA-DPA1 or one HLA-DPB1 allele mismatch. All the selected patients were diagnosed with chronic myelogenous leukemia in first chronic phase and received T-cell-replete marrow grafts following a myeloablative preparative regimen. The majority of the patients (97.4%) received calcineurin inhibitor-based GVHD prophylaxis with methotrexate. One patient received no GVHD prophylaxis. aGVHD grades 0–IV were defined by the Glucksberg scale (13).

Seven of the eight previously described *TNF* alleles (14) and one novel allele (Table S1, Supporting information) were found in the HSCT pairs. *TNF* alleles p001 (65.8%), p002 (11.9%) and p006 (10.5%) were commonly observed (Table 2). It should be noted that *TNF* and *LTA* are MHC-encoded genes lying between the class I and class II coding regions. The pairs for this study were selected based on a high degree of HLA allele match (at least 15/16) and likely predominantly carry common White HLA haplotypes. As a result, the *TNF* and *LTA* allele frequencies found in this study probably are skewed *vs* a normal randomly selected population. Interestingly, although the patients and their donors are highly HLA matched and also should be matched for *TNF* and *LTA* alleles, there were four HSCT pairs that had a *TNF* and/or *LTA* allele mismatch (data not shown). The novel allele TNFp009 (GenBank accession no. EU338455) was present in four White individuals and is similar to allele p002 (Table S1, Supporting information). Both alleles encode the SNP 2308A (database of SNP; dbSNP rs#1800629), but allele p009 also contains a cytosine insertion at +71 (dbSNP rs#4645838) in the 5′ untranslated region (UTR) of exon 1. No novel SNPs were identified in any of the donor–recipient pairs.

One study has linked the *TNF* – 308A polymorphism that is associated with high TNF production with the development of high-grade aGVHD (15). Immune system cells, especially macrophages, are the principal source of TNF. As some immune system cell types survive the conditioning regimen, it is possible that either the recipient or the donor *TNF* genotype might be associated with aGVHD development. However, in this small study of HSCT pairs, no significant differences or trends were noted in the distribution of the *TNF* −308 SNP or any other *TNF*SNP [−1031T/C (dbSNP rs#1799964), −863C/A (dbSNP rs#4645836), −857C/T (dbSNP rs#1799724), −376G/A (dbSNP rs#1800750) and −238G/A (dbSNP rs#361525)] and aGVHD development among either recipient or donor genotypes (Table 3 and data not shown). These data are consistent with other studies that showed no associations between *TNF* SNPs and development of aGVHD (12,16–18). Examination of *TNF* allele genotypes between the aGVHD-grade groups in the donors and in the recipients revealed no significant differences in distribution (Table 4). A previous study had found a significant association with aGVHD (grades III–IV) when donors or recipients were positive for the p003 (U02) and p006 (U03) TNF alleles (as defined by the combination of polymorphisms at positions −1031, −863 and −857) (19). Such an association was not present in this study as these alleles were equally distributed among the aGVHD-grade groups in both the donors [2/20 *vs* 1/18 (TNFp003) and 4/20 *vs* 3/18 (TNFp006), grades 0–I *vs* III–IV, respectively] and the recipients [1/20 *vs* 1/18 (TNFp003) and 4/20 *vs* 3/18 (TNFp006), grades 0–I *vs* III–IV, respectively] (Table 4).

Eight of the 16 previously described *LTA* promoter alleles (14) and 4 novel alleles were found in the recipient–donor pairs (Table S2, Supporting information). *LTA* promoter alleles p001, p00201, p003, p004, p007 and p011 were observed frequently (≥5%) (Table 2). The novel allele LTAp016 (GenBank accession no. EU338448) is identical to allele p004 except for a change at SNP position +697 (A–C; dbSNP rs#2229092) and was present in three individuals. The other three novel alleles encode two novel SNPs. Alleles p017 and p018 (GenBank accession nos. EU338449 and EU338450, respectively) both were present in two individuals and encode the novel SNP – 1325T/C (dbSNP SS# 86217653) in the *LTA* regulatory region. These alleles are identical to each other with the exception of the SNP +496T/C (p017 and p018, respectively; dbSNP rs#2229094) that alters the *LTA* signal peptide (Cys13Arg). Allele p019 (present in one individual; GenBank accession no. EU338451) is identical to allele p001 but encodes the novel SNP – 981G/T (dbSNP SS# 86217652) in the *LTA* regulatory region.

To date, the limited number of studies examining *LTA* SNP associations with development of aGVHD have been restricted to the +253G/A SNP (*Nco*I restriction fragment length polymorphism; dbSNP rs#909253) (16, 20). In our study, several known and putative functional *LTA* polymorphisms were examined for an association with development of aGVHD (Table 3 and data not shown). It was observed that heterozygosity for two of the *LTA* SNPs showed a trend toward association with aGVHD development. High-grade aGVHD was more likely when the donor was +253(A + G) and +724(C + A; dbSNP rs#1041981) [5/20 (25%) *vs* 9/18 (50%) for both]. Because LT-α is primarily produced by T cells, it not surprising that the donors' *LTA* genotype might be more indicative of high-grade aGVHD development. Interestingly, one study found that *LTA* heterozygosity at position +253 correlated with increased severe toxic complications (grades III–IV) after HSCT but not with aGVHD development (20). The distribution of the *LTA* − 1051G/A (dbSNP rs#3093540) and +496T/C SNP genotypes among the aGVHD-grade groups (Table 3) indicates that these SNPs also might be of interest in a larger study. None of these observed trends achieved statistical significance. The distribution of *LTA* allele genotypes showed no significant differences between the aGVHD-grade groups (Table 4). These results were not surprising considering that the number of *LTA* allelic genotypes is large.

Five of the 14 known TGFB1 promoter alleles (21) and 3 novel alleles (each found in a single individual) were identified in the HSCT pairs (Table S3, Supporting information). TGFB1p001 (32.4%) and TGFB1p003 (53.4%) were the most frequent alleles, while the other alleles were present at a low frequency (<5%) (Table 2). Two of the novel alleles, p015 (GenBank accession no. EU338454; present in an Asian-Pacific Islander who was excluded from our analyses) and p016 (GenBank accession no. EU338452), are identical except for a novel SNP +611C/G (dbSNP SS# 86217655; p016 and p015, respectively) encoded in the *TGFB1* 5′ UTR of exon 1 (Table S3, Supporting information). Interestingly, these alleles also encode a novel linkage of the functional SNPs at −509 and +869 (−509T and +869T; dbSNP rs#1800469 and rs#1982073, respectively) not previously seen among the *TGFB1* promoter alleles (21). All four possible linkages are now represented between these two functionally significant *TGFB1* SNPs. The novel allele p017 (GenBank accession no. EU338453) also encodes a novel SNP and is most similar to allele p007. The novel SNP +931T/G (dbSNP SS# 86216654) in TGFB1p017 alters codon 31 in exon 1 from *TCC* (serine) to *GCC* (alanine), which changes the amino acid sequence of the mature protein.

The principal sources of TGF-β1 are epithelial cells and regulatory T cells. Therefore, it could be anticipated that either the donor (T cell) or the recipient (epithelial cell) genotypes could play a role in and be predictive of aGVHD development. In fact, associations previously have been shown for aGVHD development and donor or recipient genotypes for the regulatory region *TGFB1* SNP −509C/T (22) and the signal peptide *TGFB1* SNPs +869T/C and +915G/C (12, 22, 23). None of these SNPs or any other *TGFB1* polymorphism examined in the present study showed a trend for an association with development of aGVHD (Table 3 and data not shown). Examination of *TGFB1* allele genotype distributions revealed that high-grade aGVHD was less frequent when the donors had the p001/p003 genotype [5/18 (27.7%) *vs* 10/19 (52.6%)] (Table 4). Furthermore, recipients whose donors carried the genotype p003/p006 were more likely to develop high-grade aGVHD [4/18 (22.2%) vs 0/19 (0%)]. However, these observations were trends that did not reach statistical significance.

It is well established that cytokines play a pivotal role in aGVHD. The molecular mechanisms involved in development of aGVHD after allogenic HSCT can be greatly influenced by the levels of cytokine expression. For instance, increased TGF-β1 expression can suppress T-cell proliferation and induce $CD4^+$ $CD25^+$ suppressor T-cell activity (24, 25), which can protect against aGVHD development (26). On the other hand, increased levels of LT-α particularly from donor-derived T cells could over stimulate the expression of various adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) (27). ICAM-1 has been found to be overexpressed in patients suffering from GVHD, and blocking ICAM-1 has been found to ameliorate the development of aGVHD (28, 29). These data suggest that polymorphisms that impact cytokine levels should be predictive of aGVHD development. However, linking cytokine genotypes to production level differences and translating this to disease outcomes has been tenuous, likely due in part to the diversity of linkages among the SNPs that impact the expression level of each cytokine. Although this small study attempted to address this issue by examining the distribution of allelic linkages of SNPs in addition to individual SNPs, no significant associations with aGVHD development were found.

It is evident that the MHC class III region *TNF* and *LTA* genes contribute to the development of aGVHD. Interestingly, it has been found that HLA-A, -B, -C, -DRB1 and -DQB1-matched allogeneic donor–recipient pairs often are mismatched (~40–75%) for microsatellite markers in the class III region and that these mismatches are significantly associated with complications after allogeneic HSCT (30, 31). Furthermore, a polymorphism in the *HSP70-hom* gene located in the class III region between the complement and the TNF

loci was shown to be associated with aGVHD development (32). These data suggest that there are genes in the MHC other than HLA, such as the class III region *TNF* and *LTA* genes, that affect transplant outcomes. Additionally, it has been suggested that individuals who carry certain HLA types are more susceptible to aGVHD development (33, 34). One possibility is that this susceptibility might be attributed to particular *TNF* and *LTA* alleles linked to specific HLA haplotypes. In either case, these observations suggest that diversity in the *TNF* and *LTA* genes and its association with aGVHD should continue to be scrutinized.

Although significant associations were not anticipated in this pilot study, our results did indicate that some *LTA* SNP and *TGFB1* allelic genotypes were distributed unequally between aGVHD-grade groups and that a larger study might prove more informative. To further investigate the interesting trends in this pilot study, a minimum of 162 pairs (81 in each aGVHD group) would be required to have at least 80% power to detect a 20% rate increase in GVHD as significant at a two-sided 5% significance level (nQuery Advisor 6.0) (35). For cytokine allele genotype distributions, a power calculation could not be made as current software cannot accommodate the large number of genotypes, particularly with respect to LTA (19 allelic genotypes observed). Even in a best-case scenario for allele genotypes, a bare minimum of 500 pairs would be required in a full study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research is supported by funding from the Office of Naval Research N00014-04-1-0784. Additional support was provided through the National Marrow Donor Program (NMDP), the Health Resources and Services Administration #240-97-0036 and the Office of Naval Research N00014-93-1-0658 to the NMDP. Biostatistics support is provided by the Georgetown University Lombardi Cancer Center Support Grant 5P30 51008. We appreciate the resources and advice provided by the Center for International Blood and Marrow Transplant Research (study no. R2003-0057). The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Navy, the Department of Defense or the US government.

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Table 1

Study population characteristics

	Grades 0–I	Grades III–IV
Number of pairs[a]	20	18
Age at transplant (median, range)	42 years (15–55)	36 years (17–54)
Donor age (median, range)	37 years (18–47)	37 years (21–53)
Male sex (%)	11 (55)	9 (50)
HLA allele match		
16/16	13	10
15/16[b]	7	8
Karnofsky prior to transplant >90	19	18
Graft-*vs*-host disease prophylaxis		
Cyclosporin A or FK506 + methotrexate	19	18
None	1	0

HLA, human leukocyte antigen; SNP, single nucleotide polymorphism

[a] Epstein-Barr virus-transformed B-cell lines from 40 recipients of bone marrow transplants and their unrelated donors were provided by the NMDP® Research Sample Repository. The majority of the recipients and donors were self-identified as White (77); one was Hispanic, one was Japanese and one was an Asian-Pacific Islander. To alleviate the impact of racial/ethnic differences of cytokine SNP and genotype frequencies on distributions in this small pilot study, the two pairs with non-White individuals in the grades III–IV category were excluded from all analyses. These pairs were retained only for the purpose of reporting novel SNPs and alleles. These cells may be obtained from the NMDP Research Sample Repository (http://www.nmdpresearch.org/SAMPLES/samples_idx.html).

[b] Donor-recipient pairs that were not fully HLA allele matched resulted from an HLA-DPA1 or an HLA-DPB1 mismatch.

Tissue Antigens. Author manuscript; available in PMC 2011 November 14.

Table 2

Cytokine promoter allele frequencies in donor–recipient pairs

TNF (*n* = 152)		LTA (*n* = 152)		TGFB1 (*n* = 148)[a]	
Allele[b]	Number (%)	Allele[b]	Number (%)	Allele[b]	Number (%)
p001	100 (65.8)	p001	71 (46.7)	p001	48 (32.4)
p002	18 (11.9)	p00201	16 (10.5)	p002	5 (3.4)
p003	5 (3.3)	p00202	0 (0)	p003	79 (53.4)
p004	1 (0.7)	p003	8 (5.3)	p004	0 (0)
p005	2 (1.3)	p004	13 (8.6)	p005	0 (0)
p006	16 (10.5)	p005	4 (2.6)	p006	7 (4.7)
p007	0 (0)	p006	2 (1.3)	p007	0 (0)
p008	6 (3.9)	p007	18 (11.8)	p008	0 (0)
p009	4 (2.6)	p008	0 (0)	p009	0 (0)
		p009	0 (0)	p010	0 (0)
		p010	0 (0)	p011	0 (0)
		p011	12 (7.9)	p012	0 (0)
		p012	0 (0)	p013	0 (0)
		p013	0 (0)	p014	7 (4.7)
		p014	0 (0)	p015	0 (0)
		p015	0 (0)	p016	1 (0.7)
		p016	3 (2.0)	p017	1 (0.7)
		p017	2 (1.3)		
		p018	2 (1.3)		
		p019	1 (0.7)		

SNP, single nucleotide polymorphism; TNF, tumor necrosis factor.

[a] There was insufficient sample to determine the TGFB1 promoter allele sequence from one of the donor–recipient pairs.

[b] Promoter allele sequences and nomenclature are described in the Tables S1–S3, Supporting information. The frequency of alleles for each of these cytokines is not significantly different in the patients and in the donors (data not shown). The *TNF* regulatory region (−1075 to +161), *LTA* regulatory region and gene (−2434 to +743) and *TGFB1* regulatory region and exon 1 (−1825 to +1252) were amplified from genomic DNA as described (14, 21). Amplified *TNF* DNA was purified using AMPure (Agencourt, Beverly, MA), and amplified *LTA* and *TGFB1* DNA were purified using Microcon Ultracel YM-100(Millipore, Bedford, MA) as per the manufacturer's protocol. Purified *TNF* and *LTA* amplification products were sequenced with the Big Dye Terminator sequencing kit (PE Applied Biosystems, Foster City, CA) as described (14) with the following modifications: reaction volume was increased (15 μl) and included Big Dye Reagent (1 μl) and Better Buffer (5 μl) (The Ge Company, San Francisco, CA). Purified *TGFB1* amplification products were sequenced as described (21). Reactions were performed in an MJ Research PTC-225 thermocycler (BioRad, Hercules, CA, USA) as per the PE Applied

Biosystems protocol. Reactions were run on an ABI3730 automated DNA sequencer (PE Applied Biosystems) according to the manufacturer's protocol. Data were analyzed with SEQUENCHER 5.1 software (Gene Codes Corporation, Ann Arbor, MI) as described (14, 21). *LTA* alleles were established by amplification and sequencing of restriction enzyme-digested genomic DNA. This used the restriction fragment length polymorphisms (RFLP) created by the SNPsat +253G/A (*Nco*I RFLP; dbSNP rs#909253) and/or at +369G/C (BskHiKA1 RFLP; dbSNP rs#746868). Genomic DNA (1 μg) was digested in a reaction (40 μl) containing buffer (1 ×), bovine serum albumin (0.4 μl) and the appropriate restriction enzyme (4 μl) as per the manufacturer's protocol (New England Biolabs, Beverly, MA) with the exceptions that the *Nco*I digest was incubated for 5 h and the BskHiKA1 digest was incubated for 18 h. Digested genomic DNA was purified using the GeneClean Turbo Kit (MP Biomedicals, Solon, OH) as per the manufacturer's protocol with the exception that the DNA was eluted in water (80 μl). Isolated *LTA* alleles were amplified and sequenced from purified digested genomic DNA as described above. *LTA* alleles for which RFLP was not possible and *TNF* alleles, where necessary, were isolated by cloning. Amplified products were cloned into the TA cloning vector (Invitrogen, Carlsbad, CA) as per the manufacturer's protocol. Amplified products were sequenced from multiple clones as described above. Allelic genotypes reported for *TGFB1* were presumed to be a combination of previously published promoter alleles (21). *TGFB1* allelic genotypes that could not be explained as a combination of known alleles were found to be heterozygous for a 3-bp insertion at position −1550 (dbSNP rs#11466313). To establish novel allelic sequences, an allele-specific polymerase chain reaction was designed to amplify alleles with the 3-bp (AGG) insertion at position −1550. Briefly, reactions (100 μl) contained genomic DNA (300 ng), primers −1550AGG (5′-AGGGCAGGGACATGAGGAGG-3′) and 3.2 (5′-TTCTTCTGCCAGTCACTTCCT-3′) (30 pm each), Platinum Taq HiFi (7.5 U) (Invitrogen), $MgSO_4$ (2 mM), dNTPs (0.2 mM each), dimethyl sulfoxide (5%) and 10× Taq HiFi buffer (1 ×). Reactions were performed in an MJ Research PTC-225 thermocycler (BioRad) by denaturation (95°C, 10 min, 1 cycle) and amplification (95°C, 30 s; 56°C, 30 s; 68°C, 3 min; 35 cycles), followed by a final extension (68°C, 20 min, 1 cycle). Amplified DNA product was purified and sequenced as described above. In some instances, allelic sequences obtained using this method did not establish a complete allele type as some genotypes also exhibit a dimorphism (−1570A/G; dbSNP rs#2803457) upstream of the 3-bp insertion at position −1550. For these genotypes, the region from −1825 to +10 was amplified as described (21). Amplified DNA was cloned using the TOPO-TA cloning vector (Invitrogen) as per the manufacturer's protocol. Cloned DNA was sequenced as described above and combined with the data generated from the allele-specific amplification to establish the complete promoter allele sequence.

Table 3

Distribution of cytokine SNP genotypes among aGVHD-grade groups

	Donor type			Recipient type		
	aGVHD			aGVHD		
Genotype[a]	0–I	III–IV	*P* value	0–I	III–IV	*P* value
TNF (−1031)						
T/T	14	13	0.72	13	13	0.48
T/C	6	4		7	4	
C/C	0	1		0	1	
TNF (−863)						
C/C	16	15	0.66	16	15	0.66
C/A	4	2		4	2	
A/A	0	1		0	1	
TNF (−308)						
G/G	14	14	>0.99	14	14	>0.99
G/A	5	4		5	4	
A/A	1	0		1	0	
LTA (−1816)						
C/C	16	15	0.66	16	15	0.66
C/G	4	2		4	2	
G/G	0	1		0	1	
LTA (−1051)						
G/G	15	16	0.18	15	16	0.18
G/A	5	1		5	1	
A/A	0	1		0	1	
LTA (+81)						
C/C	5	5	0.52	5	5	0.52
C/A	12	8		12	8	
A/A	3	5		3	5	
LTA (+253)						

	Donor type			Recipient type		
	aGVHD			aGVHD		
Genotype[a]	0–I	III–IV	*P* value	0–I	III–IV	*P* value
A/A	12	9	0.10	11	9	0.51
A/G	5	9		7	9	
G/G	3	0		2	0	
LTA (+369)						
G/G	5	5	0.52	5	5	0.52
G/C	12	8		12	8	
C/C	3	5		3	5	
LTA (+496)						
T/T	11	12	0.22	11	12	0.22
T/C	7	2		7	2	
C/C	2	4		2	4	
LTA (+724)						
C/C	12	9	0.10	11	9	0.51
C/A	5	9		7	9	
A/A	3	0		2	0	
TGFB1 (−1550)						
AGG/AGG	5	9	0.41	7	5	0.45
AGG/nil	12	8		9	12	
nil/nil	2	1		3	1	
TGFB1 (−509)						
C/C	5	9	0.41	8	4	0.21
C/T	12	8		8	13	
T/T	2	1		3	1	
TGFB1 (−869)						
T/T	4	8	0.39	7	5	0.91
T/C	12	8		8	9	
C/C	3	2		4	4	

SNP, single nucleotide polymorphism; aGVHD, acute graft-*vs*-host disease; GVHD, graft-*vs*-host disease; LTA, lymphotoxin alpha; TGFB1, transforming growth factor beta 1; TNF, tumor necrosis factor.

[a]The numbers of donors and recipients for each *TNF*, *LTA* and *TGFB1* SNP and allele genotypes were tabulated and compared by chi-squared test using the exact *P* value as implemented in StatXact-4 (exact chi-squared test; Version 4.0.1; Cytel Software Corporation, Cambridge, MA) to accommodate small sample sizes. For all SNPs, categories were collapsed to look for dominant or recessive effects on GVHD grade. However, no trends were enhanced with this method. The full SNP classifications are presented to maximize the information provided. For the SNPs, only those known to impact expression levels and/or that showed a trend in distribution among aGVHD-grade groups are presented. The sample size was based on exploratory investigation and feasibility constraints. As an exploratory pilot study, no adjustments for significance level from multiple tests were made. At this small sample size, we considered a *P* value ≤0.10 to be of interest for examining SNPs in a larger trial.

Table 4

Distribution of cytokine allele genotypes among aGVHD-grade groups

	Donor type			Recipient type		
	aGVHD			aGVHD		
Genotype[a]	0–I (%)	III–IV (%)	*P* value	0–I (%)	III–IV (%)	*P* value
TNF			0.81			0.94
p001/p001	6 (30)	9 (50)		7 (35)	9 (50)	
p001/p002	5 (25)	3 (16.6)		4 (20)	3 (16.6)	
p001/p003	2 (10)	1 (5.6)		1 (5)	1 (5.6)	
p001/p005	1 (5)	0 (0)		1 (5)	0 (0)	
p001/p006	4 (20)	2 (11)		4 (20)	2 (11)	
p001/p008	1 (5)	1 (5.6)		1 (5)	1 (5.6)	
p002/p004	0 (0)	0 (0)		1 (5)	0 (0)	
p002/p009	1 (5)	0 (0)		1 (5)	0 (0)	
p006/p006	0 (0)	1 (5.6)		0 (0)	1 (5.6)	
p008/p009	0 (0)	1 (5.6)		0 (0)	1 (5.6)	
LTA			0.63			0.43
p001/p001	3 (15)	5 (27.7)		3 (15)	5 (27.7)	
p001/p00201	3 (15)	4 (22.2)		2 (10)	4 (22.2)	
p001/p003	1 (5)	1 (5.6)		1 (5)	1 (5.6)	
p001/p004	2 (10)	0 (0)		2 (10)	0 (0)	
p001/p005	1 (5)	0 (0)		1 (5)	0 (0)	
p001/p007	3 (15)	3 (16.6)		3 (15)	3 (16.6)	
p001/p011	2 (10)	0 (0)		2 (10)	0 (0)	
p00201/p007	1 (5)	0 (0)		2 (10)	0 (0)	
p003/p005	1 (5)	0 (0)		1 (5)	0 (0)	
p003/p018	0 (0)	1 (5.6)		0 (0)	1 (5.6)	
p004/p004	0 (0)	1 (5.6)		0 (0)	1 (5.6)	
p004/p006	0 (0)	1 (5.6)		0 (0)	1 (5.6)	

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	Donor type			Recipient type		
	aGVHD			aGVHD		
Genotype[a]	0–I (%)	III–IV (%)	*P* value	0–I (%)	III–IV (%)	*P* value
p004/p007	1 (5)	0 (0)		0 (0)	0 (0)	
p004/p011	1 (5)	0 (0)		1 (5)	0 (0)	
p007/p017	1 (5)	0 (0)		1 (5)	0 (0)	
p011/p011	0 (0)	1 (5.6)		0 (0)	1 (5.6)	
p011/p016	0 (0)	1 (5.6)		0 (0)	1 (5.6)	
p016/p019	0 (0)	0 (0)		1 (5)	0 (0)	
TGFB1			0.31			0.29
p001/p001	2 (10.5)	1 (5.6)		3 (15.8)	1 (5.6)	
p001/p003	10 (52.6)	5 (27.7)		6 (31.5)	9 (50)	
p001/p014	0 (0)	1 (5.6)		0 (0)	3 (16.6)	
p002/p003	2 (10.5)	1 (5.6)		1 (5.3)	0 (0)	
p002/p017	0 (0)	0 (0)		1 (5.3)	0 (0)	
p003/p003	4 (21.1)	5 (27.7)		5 (26.3)	3 (16.6)	
p003/p006	0 (0)	4 (22.2)		2 (10.5)	1 (5.6)	
p003/p014	1 (5.3)	1 (5.6)		1 (5.3)	0 (0)	
p003/p016	0 (0)	0 (0)		0 (0)	1 (5.6)	

aGVHD, acute graft-*vs*-host disease; LTA, lymphotoxin alpha; TGFB1, transforming growth factor beta 1; TNF, tumor necrosis factor.

[a]Statistical analysis was performed as described in the footnote to Table 3.