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Polymorphisms within the genes encoding TNF- α and TNF- β associate with the incidence of post-transplant complications in recipients of allogeneic hematopoietic stem cell transplants

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Summary

Hematopoietic stem cell transplantation (HSCT) is a curative treatment of many hematological disorders. Recent studies have shown the associations between polymorphic features of cytokine-encoding genes and the incidence of post-transplant complications in the recipients of allogeneic HSCT. This review focuses on the relationship between the polymorphic patterns of patient genes encoding tumor necrosis factor (TNF)- α and TNF- β and the manifestation of post-transplant complications, acute graft-versus-host disease (aGvHD), generation of toxic lesions, and mortality. Discussed in more detail are the relationships of TNFd microsatellites and polymorphisms within the promoter region of the TNF- α -encoding gene (*TNFA*) in the position (–308) and within the first intron of the TNF- β -encoding gene (*TNFB*). It appeared that heterozygosity within the *TNFA* promoter and the first intron of the *TNFB* gene increased the susceptibility to severe grades III–IV of toxic complications, while the presence of the TNFd3 homozygous genotype was associated with a higher risk of severe aGvHD and early mortality in patients after allogeneic HSCT. These results imply that donor-recipient genotyping, extended to cytokine loci, may be of prognostic value for transplantation outcome.

Key words: allogeneic HSCT • TNF polymorphism • GvHD • toxicity

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INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a curative treatment for many hematological disorders. Siblings compatible with the recipient with regard to both human leukocyte antigen (HLA) haplotypes are optimal for blood cell or bone marrow donation. However, acute graft-versus-host disease (aGvHD) still remains a major complication, even in the best HLA-matched donor-recipient pairs, affecting up to 40–50% of patients. The high occurrence in this group of patients is largely due to minor histocompatibility differences between donor and recipient³⁴, and the presence of particular HLA specificities in donor-patient pairs^{6, 22, 75}. More recently, non-HLA genes encoding cytokines involved in the pathogenesis/control of the disease have been implicated^{56, 57}. These observations have led to studies investigating the possible role of patient cytokine genotypes that may influence the development of aGvHD through cytokine activation during the pre-transplant conditioning regimens. Recently published data have documented the associations of polymorphic features of cytokine genes with post-transplant complications in allogeneic HSCTs^{8, 9, 19, 20, 43, 45, 49, 56, 70}. These have included tumor necrosis factor (TNF)- α and TNF- β cytokines encoded within the major histocompatibility complex (MHC) on chromosome 6, as well as those with genes located on other chromosomes, as, for example, interferon (IFN)- γ , interleukin (IL)-6, IL-10, and transforming growth factor (TGF)- β . Cytokine genotypes associated with aGvHD are listed in Table 1. It has been documented that TNF and IL-10 microsatellites^{19, 56}, and single nucleotide polymorphisms of the IL-10, IFN- γ , and TGF- β -encoding genes^{56, 57}, associate with the risk for aGvHD manifestation. It has also been observed that TNF- α gene (*TNFA*), TNF- β gene (*TNFB*), and IL-6 gene polymorphisms associate with the susceptibility for severe toxic complications^{8, 42, 43}.

Table 1. Polymorphic features of cytokine-encoding genes associated with increased risk of aGvHD in the recipients of allogeneic HSCT

Polymorphism	Genetic marker	Reference
TNF microsatellite	d3/d3 homozygotes	56
IFN- γ (+874 T/A)	allele A	44
	T/A heterozygotes	49
IFN- γ (intron 1)	homozygotes with allele 3 ^a	19
IL-6 (-174 C/G)	allele G ^b	44
IL-10 (-1064) (CA) _n	(CA) _{≥12}	19, 56
IL-10 (-1082 G/A, -819 C/T, -592 A/C)	GCC/GCC homozygotes	65
	GCC	42
	allele G	42, 44

^a Allele 3 of the I intron of IFN- γ gene also associates with chronic GvHD incidence⁶².

^b IL-6 (-174) G/G homozygosity is a marker of increased risk for chronic GvHD¹⁹.

Therefore, there are a number of cytokines whose polymorphic features may contribute to the development of post-transplant complications. This paper is focused on associations between TNF alleles of recipients of allogeneic stem cells and transplant outcome, the generation of toxic lesions, and the incidence of aGvHD and mortality.

ROLE OF TNF- α IN THE PERPETUATION OF GvHD

GvHD occurs when transplanted donor T lymphocytes react to foreign host cells and it is characterized by hematopoietic dysfunction, immunosuppression, and a wide variety of host tissue injuries. The skin, gastrointestinal tract, and liver are major target organs of aGvHD. Both T cells and cytokines, including TNF- α , are the major effectors in GvHD. The initial phase of GvHD is arguably initiated prior to transplantation, where necessary transplant conditioning regimens damage GvHD target organs (initially skin and gut). This conditioning gives rise to subsequent release of cytolytic cytokines, including TNF- α and IL-1. This in turn causes an upregulation of HLA-DR⁴¹ and adhesion molecule expression, which aids to activate allogeneic donor T cells during the initial phase following stem cell transplantation. Proliferation of T cells, release of IL-2, and the cytokine cascade or “storm” ensue, causing the characteristic lesions associated with GvHD in the target organ⁵². Acute GvHD usually commences 30–100 days post-transplant and is the most studied form of GvHD. Chronic GvHD (cGvHD), occurring, by definition, more than 100 days post-transplant, is often, but not always, associated with prior aGvHD and can in most respects be defined as a separate disease with autoimmune type associations²¹.

TNF- α and TNF- β are pro-inflammatory cytokines with a number of biological activities, including the mediation of inflammatory responses³². Pro-inflammatory cytokines, especially TNF- α , are involved in the induction and effector phase of aGvHD^{3, 31}. TNF- α is released pre-transplant due to irradiation and cytotoxic pre-conditioning regimens. TNF- α serum level during the pre-conditioning treatment has been shown to correlate with severe complications, the manifestation of aGvHD, and mortality after HSCT³⁶.

The role of TNF- α as a mediator of graft-versus-host reactions (GvHR) has also been analyzed by an *in vitro* skin explant assay^{13, 26, 28}. This assay involves sensitizing donor lymphocytes *in vitro* in a primary mixed lymphocyte reaction (MLR) and then evaluating the secondary response on patient skin biopsies by grading the GvHR (grades I–IV) histopathologically. The

in vitro skin explant model has been successfully used to predict aGvHD in HLA-identical sibling bone marrow transplants^{25, 27, 30, 67} and to investigate the role of cytokines (IFN- γ and TNF- α) in GvHR^{26, 28}. It has been observed that unmatched mixed lymphocyte culture supernatants containing high levels of TNF- α could produce a graft-versus-host type of histopathological damage in human skin explant assays. TNF- α production in an *in vitro* skin explant assay has been found to correlate with the development of more severe GvHD^{26, 28}. Extended studies, including an analysis of cytokine production of T cell lines and clones derived from the skin explant culture, have also implicated a role of TNF- α as a factor affecting the manifestation and perpetuation of GvHD in recipients of allogeneic bone marrow transplants (BMT)^{5, 13}.

POLYMORPHISM WITHIN TNF-ENCODING GENES

The TNF genes are located within the class III region of the human MHC, telomeric to the HLA class II and centromeric to the HLA class I region (Fig. 1). This region consists of at least 3 functional genes encoding TNF- α , TNF- β (also known as lymphotoxin (LT)- α), and LT- β ¹⁸. Genes of the TNF locus map at ~1000 kb telomeric to the DR and ~1300 kb centromeric to the B locus in the human MHC.

In humans, genes located within the MHC, especially HLA class I and class II loci, characterize by enormous polymorphism⁶³. A number of genetic markers have also been identified for genes that map at the TNF locus. One of the first, a dimorphism within the

first intron (+1069) of the *TNFB* gene, has been described^{55, 66}. *TNFB**1 (*TNFB*1) and *TNFB**2 (*TNFB*2) alleles characterize individuals carrying C or T in position +1069 within the first intron of *TNFB* gene (correlating with the presence of either asparagine or threonine in position 26, respectively⁵⁵).

Genetic analyses have also revealed a G to A transition polymorphism at position –308 in the promoter/enhancer region, an AP-2 transcription binding site, of the *TNFA* gene⁷⁹. *TNFA**1 (*TNF*1) and *TNFA**2 (*TNF*2) alleles are carried by individuals having, respectively, G or A at the –308 polymorphic position of the *TNFA* promoter.

In addition to the (–308) polymorphic site, the *TNFA* gene is characterized by other nucleotide substitutions in the next 8 positions: +70 (G/A)¹⁶, –1031 (T/C)³⁵, –163 (G/A)¹⁶, –238 (G/A)²³, –308 (G/A)⁷⁹, –376 (G/A)¹⁶, –574⁷⁴, –856 (C/T)^{35, 74}, and –862 (C/A)^{35, 74}.

Polymorphism within the *TNFA* and *TNFB* genes (–308 and +1069, respectively) can be analyzed by polymerase chain reaction (PCR) amplification followed by *Nco*I digestion of the obtained amplifications^{11, 66, 79}. In the case of *TNFA* –308 promoter polymorphism, this is indirect *Nco*I restriction fragment length polymorphism typing. The *Nco*I restriction site for *TNFA* is induced into the amplification product of the allele with G in the position –308 by the 5'-primer⁷⁹.

In brief, with the use of specific primers (*TNFA*: 5'-AGGCAATAGGTTTGTAGGGGCC AT-3' and 5'-TCCTCCCTGCTCCGATTCCG-3'; *TNFB*: 5'-CCGTGCTTCGTGCTTTGGAC TA-3' and 5'-AGAGGGGTGGATGCTTGGGTTTC-3'), the 107 bp (with induced *Nco*I restriction site) and 782 bp fragments are amplified for the promoter region of the *TNFA* gene and the first intron of the *TNFB* gene, respectively. Then, PCR products are digested with *Nco*I restriction enzyme and analyzed on agarose gel. For visualization of the *TNFB* and *TNFA* alleles, 2 and 4% gels are used, respectively. Electrophoresis demonstrates the original 107 bp fragment (homozygous individuals *TNFA**2 allele, lacking the *Nco*I site), three fragments of 107, 87 and 20 bp of length (heterozygous individual), and two fragments of 87 and 20 bp (homozygous individual for the allele *TNFA**1). A similar pattern is seen for the *TNFA* alleles. The restriction digests of the *TNFB* amplifications generate fragments of 586 and 196 bp or 782 bp for *TNFB**1 or *TNFB**2 homozygous individuals, respectively. For heterozygous individuals, three fragments (196, 586 and 782 bp) are detected.

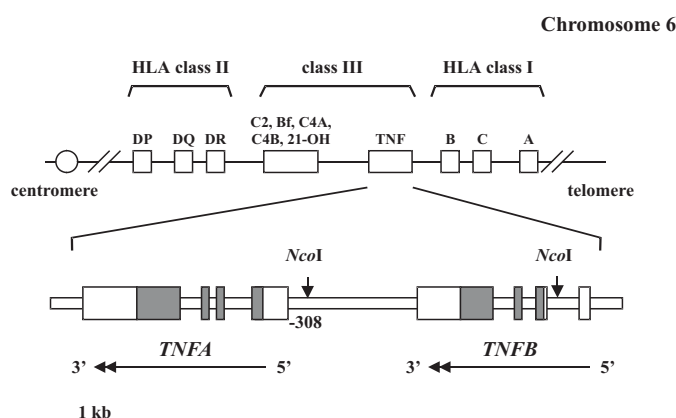


Figure 1. Genes within the human MHC with more detailed structure of the TNF gene region. Open boxes represent the relative positions of the indicated loci. Underneath is a detailed representation of the TNF gene region. Open and closed boxes indicate untranslated and translated portions of the TNF exons, respectively. Double arrows indicate the transcriptional orientation of the genes. Single arrows show an *Nco*I restriction sites. The –308 polymorphic position within *TNFA* gene is also indicated.

Table 2. Frequencies of TNF alleles in healthy individuals and patients with hematological disorders

	TNFA*1	TNFA*2	TNFB*1	TNFB*2	Number of cases	References
Healthy individuals	0.85	0.15	0.31	0.69	130	5
	0.81	0.19	0.35	0.65	216	64
	0.85	0.15	0.24	0.76	124	4
	0.84	0.16	0.26	0.74	117	24
	0.86	0.14	–	–	51	77
	0.84	0.16	–	–	40	53
Patients with hematological disorders	0.89	0.11	0.30	0.70	125^a	5
	0.945	0.055	0.26	0.74	73 (CLL)	24
	0.81	0.19	0.38	0.62	76 (B-CLL)	53
	–	–	0.33	0.66	54 (NHL)	53
	0.82	0.18	–	–	49 ^a	56
	0.78	0.22	–	–	49 (CLL)	77
	0.85	0.15	–	–	36 (HD)	77

CLL – chronic lymphocytic leukemia, NHL – non-Hodgkin's lymphoma, HD – Hodgkin's disease.

^a Recipients of allogeneic HCST suffered from different hematological disorders.

Original results are given in bold. All the patients included in this study are of Polish origin and were transplanted at the Lower Silesian Center for Cellular Transplantation with the National Polish Bone Marrow Donor Registry (formerly BMT Unit, K. Dłuski Hospital) in Wrocław.

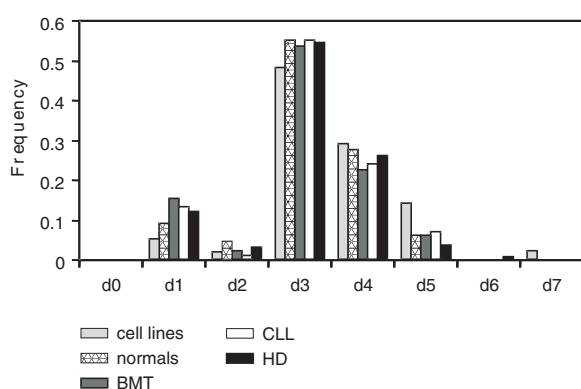


Figure 2. Frequencies of TNF microsatellite alleles based on the data of: 80 bone marrow transplant (BMT) recipients, 28 unselected normals⁵⁶, 57 patients with chronic lymphocytic leukemia (CLL), 100 patients with Hodgkin's disease (HD)⁵³, and a panel of 105 cell lines from the American Society of Histocompatibility and Immunogenetics Workshop and the Center for Human Polymorphism Studies reference panels of HLA typing cell lines⁷³.

Table 2 shows the allele frequencies of the TNFA and TNFB loci in a panel of randomly selected normal healthy individuals. The TNFA*1 allele, in contrast to the TNFA*2, is the most frequent TNF allele, present in over 85% of all individuals studied. TNFB*2 is rarer than TNFA*1, but occurs more frequently than the TNFB*1 allele.

Besides *NcoI* alleles, also a number of microsatellites have been mapped within the TNF locus⁷³. These short repetitive sequences occasionally appear not only in non-coding regions, but within the coding regions of particular genes as well. They may function in different ways, which are currently not well understood. So far, 5 different TNF microsatellites have

been identified^{58, 73}: TNFa(2), b(1), c, d, e. One of these in particular, TNFd, a (GA)_n repeat microsatellite sequence⁷³, has been mapped downstream of the TNF- α encoding gene within intron 3 of the near-by leukocyte-specific transcript-1 gene³⁷.

The presence of TNF microsatellites can be detected by using unique primers and PCR to amplify the microsatellite-containing region and measuring the length of the amplified fragments. Alternatively, amplified DNA fragments can be analyzed by sequencing^{58, 73}.

According to the summarized data^{53, 56, 73}, among the TNFd microsatellites TNFd3 is the most frequent, then d4 and d1, followed by d5, d2, while d6 and d7 occur very rarely (Fig. 2).

TNF- α AND TNF- β PRODUCTION IS INFLUENCED BY POLYMORPHIC PATTERNS OF CYTOKINE-ENCODING GENES AND HLA

TNF- α and TNF- β secretion in relation to TNF genotypes

Polymorphism at the TNF gene cluster has been shown to be associated with variations in TNF- α and TNF- β production. Pociot et al.⁶¹ found that individuals homozygous for TNFB*2 secrete higher amounts of TNF- α than do heterozygous individuals and individuals homozygous for TNFB*1 allele. This was shown for phytohemagglutinin- and lipopolysaccharide-stimulated monocytes. Also patients with severe sepsis who were homozygous for TNFB2 displayed higher circulating TNF- α concentrations than did heterozygous (TNFB1/TNFB2) individuals⁶⁶.

Bouma et al.¹⁴ documented that individuals carrying the *TNFA**2 allele secrete higher levels of TNF- α . It has been suggested that high levels of TNF- α secretion after mitogen stimulation of peripheral blood lymphocytes from BMT recipients may be associated with the *TNFA**2 allele⁵⁴. The increased *TNFA* gene expression related to the *TNFA**2 allele has also been reported by other groups (i.e.^{38, 47, 48, 80}). However, there is some conflicting data which suggests that *TNFA* (–308) polymorphism has no effect on *TNFA* gene expression^{17, 33, 39, 71, 74}.

Similar to some reports on *TNFA* polymorphism, it has been suggested that the polymorphic pattern of the *TNFB* gene also correlates with the TNF- β response of mitogen-stimulated peripheral blood mononuclear cells (at the mRNA and protein level)⁵⁵. It has been shown that the level of TNF- β secreted correlates with the *TNFB* genotype in healthy individuals: those with the *TNFB**2 allele secreted significantly higher levels of TNF- β than those with the *TNFB**1 allele. In insulin-dependent diabetes mellitus (IDDM) patients, the reverse situation was observed⁷⁶.

In addition to the associations described for *TNFA* and *TNFB* *Nco*I alleles, some relationships have also been found between the presence of particular TNF microsatellites and TNF- α and TNF- β production. The *TNFD3* microsatellite allele has been reported as being associated with higher *in vitro* TNF- α production in cells derived from immunosuppressed heart transplant recipients⁷¹. *TNFA6* has been correlated with decreased TNF- α secretion, while *TNFA2* has been associated with increased TNF- α secretion⁶⁰. Among IDDM patients, individuals carrying either *TNFA2* or *TNFA9* have presented with greater TNF- α production, while those positive for *TNFA13* have lower TNF- α production than patients with non-*TNFA2*, *a9*, and *a13* alleles⁵⁹.

Relationships between TNF alleles and HLA class I and II loci and their effect on TNF- α and TNF- β secretion

The *TNFA* and *TNFB* genes are located within the human MHC complex, between the HLA class I and HLA class II loci (Fig. 1). This close localization favors associations between HLA class III (TNF) and other HLA loci. Therefore, some relationships between the polymorphic pattern of TNF-encoding genes and the presence of particular HLA class I and/or HLA class II specificities have also been identified (please note Table 3). For example, very strong associations have been shown for the A1 B8 DR3 DQ2 haplotype with a rare *TNFA**2 allele and

Table 3. Association between HLA and TNF alleles

Haplotypes	References
A1 B8 DR3 <i>TNFA</i> *2	2, 78
A1 B8 DR3 <i>TNFA</i> *2 DQA1*0501 DQB1*0201 <i>TNFA</i> *2 A <i>TNFB</i> *1 (5,5)	15
A2 B44 <i>TNFA6</i> <i>TNFB</i> *1 DR4 DQ8 A2 B15 <i>TNFA2</i> <i>TNFB</i> *2 DR4 DQ8 A2 B40 <i>TNFB</i> *1 DR6 A1 B8 <i>TNFB</i> *1 DR3 DQ2	60
B18 <i>TNFB5</i> <i>TNFA1</i> <i>TNFB</i> *2 <i>TNFC2</i> DRB1*0301 DQA1*0501 DQB1*0201 DQB1*0201 DQA1*0501 DRB1*0301 <i>TNFC2</i> <i>TNFB</i> *2 <i>TNFA1</i> <i>TNFB5</i> B18	62
Bw62 DR4 <i>TNFB</i> *2	76
<i>TNFA</i> *1 <i>TNFB</i> *2 <i>TNFA</i> *2 <i>TNFB</i> *1 <i>TNFA</i> *2 <i>TNFB</i> *1 DR3 <i>TNFA</i> *1 <i>TNFB</i> *1 DR1 <i>TNFA</i> *1 <i>TNFB</i> *2 DR6 and/or DR7	11, 12

Table 4. Markers associated with increased expression of TNF- α -encoding gene

	Genetic marker	References
<i>Nco</i> I alleles	<i>TNFA</i> *2	14, 38, 47, 48, 54, 68, 80
	<i>TNFB</i> *2,2	61, 66
Microsatellite	<i>TNFA2</i>	59, 60
	<i>TNFA9</i>	59
	<i>TNFD3</i>	71
HLA-DR	DR3	1, 40, 60
	DR4	40, 60

between this *TNFA* allele and *TNFB**1. These in turn have been shown to affect TNF production. It has been documented that TNF- α secretion could be influenced by HLA-associated features; for example, with high secretion potential among DR3 and DR4 and low among DR5 normals⁶⁰ (Table 4). Inducibility of TNF- α has also been found to be low for DR2-positive systemic lupus erythematosus patients and high for those having HLA-DR3 and DR4 genotypes⁴⁰. The results of other study have demonstrated higher TNF- α activity in culture supernatants of (A1, B8, DR3) lymphoblastoid cell lines than that present in the supernatants from cells homozygous for eight different MHC ancestral haplotypes¹ (Table 4).

TNF POLYMORPHISM AND HSCT OUTCOME

*Nco*I *TNFA* (–308) and *TNFB* (+1069) polymorphisms do not affect development of aGvHD

Very early studies from our research group suggested that (–308) polymorphism within the *TNFA* promoter region might influence the manifestation of

aGvHD in alloHSCT recipients^{7,10}. In these analyses, 39 recipients of HLA-matched sibling transplants and, for comparison, 51 healthy individuals were investigated. It was found that patients were more frequently *TNFA**1,1 than controls and that all the patients with fatal aGvHD complications were homozygous for *TNFA**1. Patients rarely had the *TNFA**1,2/*TNFB**1,2 genotype, and an association of *TNFA**1,1 with *TNFB**1,1 was seen only in 3 out of 39 patients, but not in controls. Furthermore, all patients carrying the *TNFA**1,1 *TNFB**1,1 genotype suffered from aGvHD.

In concordance with this data, a study by Mayer et al.⁵⁴ on 53 chronic myeloid leukemia patients receiving allogeneic BMT also indicated a possible role of *TNFA* –308 polymorphism in severe GvHD due to the increased secretion of TNF- α .

The first studies on *TNFB* gene polymorphism in recipients of allogeneic HSCT reported a higher incidence of *TNFB* heterozygous cases among patients with aGvHD, especially in those who received aggressive chemotherapy (receiving thio-tepa or etoposide in addition to busulphan and cyclophosphamide)^{50, 51}. However, our further analyses have not confirmed these observations^{8,9} of an association between TNF polymorphisms of the recipient and aGvHD incidence. No relationship has been observed between either *TNFA* (–308) and/or *TNFB* (+1069) polymorphism and the manifestation of aGvHD complications^{8,9}. This concurred with other published data, as a lack of association between *TNFA* polymorphism in the (–308) position and incidence of aGvHD in allogeneic HSCT recipients has also been reported by other groups^{54, 56, 70}. In our more recent studies, *TNFA* and *TNFB* alleles have shown similar distributions in patients and control individuals. A higher rate of *TNFA**1 homozygotes has been found to characterize patients and controls, but (in contrast to the previous data) without significant difference between both groups (not shown). In both patients and healthy individuals, *TNFA**1 appeared as the greatest and *TNFB**2 as the least frequently seen of the *TNFA* and *TNFB* alleles⁵ (Table 2). Comparable frequencies of TNF alleles in hematological patients have been documented in other studies (Table 2).

Microsatellite polymorphism within the gene encoding TNF- α and the incidence of aGvHD and early mortality

As discussed above, no association has been found between *TNFA* polymorphism in the –308 position and manifestation of aGvHD. However, the studies

by Middleton et al.⁵⁶ and Kogler et al.⁴⁶ have documented the association of aGvHD (grades III–IV) complications post hematopoietic stem cell transplantation with the presence of the d3 homozygous allele in bone marrow and cord blood transplant recipients, respectively. In more detail, the *TNFD3* homozygous allele has been documented as being preferentially associated with more severe (III–IV) GvHD grades (in 7 out of 11 patients) compared with its occurrence in 8 out of 38 patients with no or mild GvHD (grades 0–II)⁵⁶. In this study, 49 patients transplanted from HLA-matched sibling donors using only cyclosporine A monotherapy were investigated. Interestingly, results of this analysis were also correlated with *in vitro* skin explant results in 32 of the 49 patient donor pairs tested²⁷. The d3 allele of the *TNFD* microsatellite preferentially associated with the more severe GvHD grades III–IV and with positive (grade II or above) skin explant assays. However, these observed relationships between *TNFD3* and GvHD outcome were not seen in an extended study involving a larger group of BMT recipients (144 cases) receiving both cyclosporine A and metotrexate prophylaxis. In this group of patients, the presence of *TNFD3* was found to correlate with early mortality²⁰. More *TNFD3*/d3-positive patients died before day 30 than non-*TNFD3*/d3 recipients. In addition, the presence of *TNFD3*/d3 homozygotes, associated with the risk of transplant-related complication in HSCT patients, has been also shown to affect organ transplants, for example to associate with increased rejection in cardiac transplant recipients⁷².

TNFA (–308) and TNFB (+1069) polymorphism and manifestation of toxic complications

Relationships between cytokine gene polymorphisms and the generation of severe toxic lesions have been extensively analyzed by our research group and, in fact, all results known so far come from our studies^{8,9, 49–51}. Our first observations suggesting a possible influence of *TNFA* heterozygous genotypes on the manifestation of severe toxic lesions in patients after allogeneic HSCT were presented in 1999 during the 25th Annual Meeting of the European Group for Blood and Marrow Transplantation (Hamburg, Germany)⁵⁰ and the 13th Meeting of the European Federation for Immunogenetics (Crete, Greece)⁵¹. In these studies, 72 and 97 recipients of allogeneic stem cells were investigated, respectively. It was found that in patients on standard conditioning regimen a high degree of toxicity was present more frequently in *TNFA**1,2-positive individuals. In the next analyses, homozygous patients carrying the *TNFB**2 allele were found to suffer less frequently from severe (grades III and

IV) toxic complications⁴⁹. Please note that in all our studies regarding the susceptibility to toxic complications, toxicity was evaluated according to the World Health Organization standard criteria (for detail see: www.fda.gov). The highest grade of toxic complications seen in any of the evaluated organs (mucosa, skin, liver, and gut) was regarded as the representative value of toxicity seen in an individual patient.

The extended studies^{8,9} proved our preliminary data on the influence of TNF polymorphic features on the generation of severe toxic lesions in recipients of allogeneic HSCT. A higher incidence of toxic complications was seen among patients heterozygous for *TNFA* and/or *TNFB*, while lower in those carrying the *TNFB**2,2 homozygous genotype. In the first of these studies, patients with hematological malignancies grafted from sibling, alternative, or matched unrelated donors were analyzed. Recipients carrying the *TNFB**2 homozygous genotype suffered less frequently from severe grades II–IV and grades III–IV toxic complications, while those having the *TNFA**1,2 and/or *TNFB**1,2 genotype were found to be more susceptible to developing severe (grade III–IV) toxic lesions⁸.

More detailed analysis focused on a group of patients with hematological malignancies, recipients of HLA-matched sibling transplants, were concordant with the previous results. Also in this cohort of patients, *TNFA**1,2 and *TNFB**1,2 were found to be associated with increased risk of developing severe grades III–IV toxic lesions, while *TNFB**2 and *TNFA**1 homozygosity had a protective role (Fig. 3). This

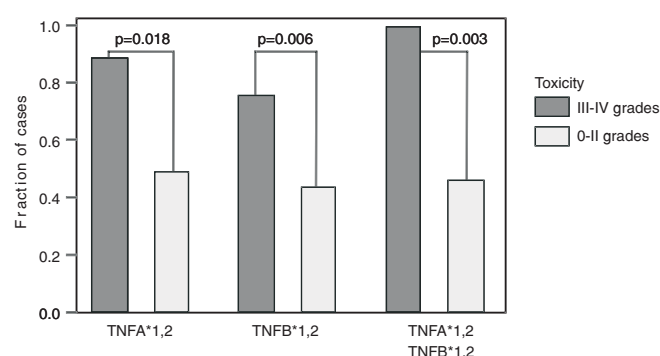


Figure 3. Manifestation of grades III–IV toxic complications in alloHSCT recipients in relation to TNF genotypes. There is an increased frequency of patients presenting with severe toxic complications among heterozygous individuals carrying *TNFA**1,2 (9/10 vs. 30/60, $p < 0.05$), *TNFB**1,2 (20/26 vs. 19/44, $p < 0.01$) and *TNFA**1,2 *TNFB**1,2 (9/9 vs. 30/61, $p < 0.005$)⁹. Toxicity was evaluated according to the World Health Organization standard criteria. The highest grade of toxic complications seen in any of the evaluated organs (mucosa, skin, liver, and gut) was regarded as the representative value of toxicity seen in the individual patient.

effect was independent of the severity of the conditioning regimen. The associations between TNF heterozygotes differed from those characterizing *TNFA**1 and *TNFB**2 homozygous patients. It has been shown that the two latter genotypes (*TNFA**1,1 together with *TNFB**2,2), in a combined associated fashion, play a protective role, while *TNFA**1,2 in *TNFB**1,2-negative individuals and *TNFB**1,2 in those having *TNFA**1,2 influence the development of severe (grades III–IV) toxic lesions^{5,9}.

Interestingly, our preliminary study regarding the outcome of alternative (HLA haplo-identical and matched unrelated) transplants have suggested that in HLA-A, -B, -DR matched alternative settings differences in TNF alleles may influence the risk of aGvHD⁵. This study involved 25 patient-donor pairs. It appeared that lack of HLA-A, -B, -DR matching between patients and donors did not affect matching of *TNFA* and *TNFB* alleles. Only in 1 out of 7 cases where patients and donors were not matched at one of the HLA class I or II loci (A, B, DR) were differences in *TNFA* and *TNFB* polymorphic features detected. Among A-, B-, DR-matched patients-donor pairs, typing results at TNF loci were discrepant in 7 cases. In 5 out of these 7 cases, patients developed aGvHD (grade II–IV). The incidence of aGvHD complications in alternative settings were more frequent in A-, B-, DR-matched, TNF-mismatched transplants than in A-, B-, DR-, TNF-matched transplants (0.71 vs. 0.27, $p = 0.088$, Fig. 4). Obviously, these results need to be confirmed on larger cohorts of donor-recipient pairs.

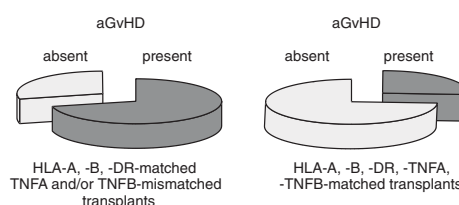


Figure 4. Influence of TNF incompatibility on the incidence of grades II–IV acute GvHD in HLA-A, -B, -DR-matched alternative HSCT. A higher incidence of aGvHD was observed among patients undergoing HLA-A, -B, -DR-matched TNF-mismatched transplants than those grafted from HLA-A, -B, -DR, TNF-matched donors (0.71 vs. 0.27, $p = 0.088$).

CONCLUSIONS

It has been shown that cytokine gene polymorphisms, in particular alleles at the TNF loci, affect the incidence and severity of post-transplant complications. Thus, the pre-transplant analysis of the patients'

genetic predisposition may be considered an important factor allowing the classification of the transplant recipients as less or more susceptible to developing either toxic lesions or severe and/or fatal aGvHD. Therefore, patient-donor genotyping, extended to the cytokine loci, may be of prognostic value for the transplantation outcome (for the generation of toxic lesions or the course of GvHD, as

shown, for example, for HLA-DR and TNF- α or HLA-DR and IFN- γ in other disease^{69, 81}). These results could then be used to tailor GvHD prophylaxis and/or to help in donor selection when more than one sibling or matched unrelated donor is available, together with aiding the development of therapeutic strategies, possibly with the use of anti-cytokine therapies or prophylaxis.

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