Brief report

Interleukin-10 and tumor necrosis factor alpha region haplotypes predict transplant-related mortality after unrelated donor stem cell transplantation

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Certain cytokine gene polymorphisms have been shown to correlate with outcome of human leukocyte antigen (HLA) identical sibling donor stem cell transplantation (SCT), but in unrelated donor SCT such information is scarce. We have studied the association between cytokine gene polymorphism and transplant-related mortality (TRM) in 182 unrelated SCTs performed at a single center. We found association of polymorphism in the tumor necrosis factor alpha (TNFα) and interleukin-10 (IL-10) gene and TRM. Both the TNFd4 allele and the TNFα −1031C alleles are associated with high risk for TRM. Statistical analysis showed that both polymorphisms were present on a single haplotype. This haplotype was associated with high risk of TRM when present in recipient or donor, 55% (43%-67%) compared with 21% (12%-30%) when absent from both (*P* < .01). A further allele associated with this haplotype, TNFa5, is also associated with increased risk of TRM. For IL-10, presence of the donor R2-G-C-C haplotype was associated with decreased risk of TRM, 61% (43%-79%) versus 34% (25%-43%), *P* = .01. In contrast, possession of the R3-G-C-C haplotype by the donor predicted reduced risk of TRM, 30% (19%-41%, 95% CI) versus 53% (40%-66%, 95% CI), *P* = .01. No independent associations of cytokine polymorphisms with acute graft-versus-host disease were shown. (Blood. 2004;103:3599-3602)

Introduction

Tumor necrosis factor alpha (TNFα) is a member of the TNF receptor superfamily of ligand receptors and, as a T-helper cell 1 (Th1) cytokine, is an important component of the proinflammatory immune response. Intensive chemotherapy and irradiation given prior to stem cell transplantation (SCT) is associated with increased serum levels of TNFα.[1-3] High levels of serum TNFα measured before transplantation are associated with increased probability of early transplant-related mortality (TRM) and acute graft-versus-host disease (aGVHD).[2,4]

Interleukin-10 (IL-10) is a member of an expanding family of cytokines known as the interferon receptor superfamily.[5] It is an important anti-inflammatory cytokine in a number of immune pathways. A reduction in TRM after SCT has been associated with increased recipient levels of IL-10 immediately before and after transplantation; however, this observation remains controversial.[4,6,7]

The genes encoding TNFα (*TNFSF2*) and IL-10 (*IL10*) are both highly polymorphic. The *TNFSF2* gene maps to chromosome 6 (6p21.3) and contains a number of single nucleotide polymorphisms (SNPs), predominantly in the 5′ promoter region.[8] *TNFSF2* gene is surrounded by a number of other genes with immunologic functions. A number of well-studied microsatellites have been described within genes flanking *TNFSF2*.[8] TNF*a*, TNF*b*, and TNF*c* microsatellites are found in or near the lymphotoxin alpha gene, *TNFSF1*. TNF*d* and TNF*e* microsatellites are located in intron 3 of the leukocyte-specific transcript gene, *LST1*. The *IL10* gene maps to chromosome 1 (1q31-32). The 5′ promoter region of this gene contains a number of proximal[9] and distal[10] SNPs as well as 2 microsatellites termed IL-10R and IL-10G.[11,12] In both *TNFSF2* and *IL10* genes investigators have sought polymorphic profiles that can be defined as haplotypes.[11,13,14]

Polymorphisms in the *TNFSF2* region and *IL10* genes have been associated with clinical outcome in several identical sibling stem cell transplantation (ID-SCT) studies.[12,15,16] However for unrelated donors (UD-SCT), very few studies have been undertaken.[17] Therefore, we investigated the impact of *TNFSF2* and *IL10* polymorphisms on TRM after UD-SCT by analyzing individual polymorphisms and statistically inferred haplotypes. We have identified polymorphic haplotypes in the *TNFSF2* region and in *IL10* that may facilitate the selection of patients and donors for UD-SCT by identifying individuals at higher risk of TRM.

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Study design

Patients

One hundred eighty-two patients undergoing UD-SCT at the University of Minnesota Hospital between 1990 and 2000 were studied. Forty-six percent of patients were younger than 18 years of age and 32% received transplants for nonmalignant disease. Sixty-nine percent of those with hematologic malignancy had early disease, acute leukemia in first or second complete remission, or chronic myelogenous leukemia (CML) in chronic phase at the time of transplantation. All donors were older than 18 years of age and the median donor age was 37 years. Sixty-five percent of transplantations were performed after 1995 and the median follow-up on survivors was 4.8 years. Eighty-nine percent of patients received total body irradiation–based pretransplantation protocols and 66% received T-cell–replete marrow transplants and received cyclosporine and methotrexate after transplantation. In 34% of cases T cells were removed by elutriation. Pretransplantation serologic typing indicated all donor-recipient pairs were matched at human leukocyte antigen A (HLA-A) and B loci. HLA-C locus typing was not performed. Sequence-specific oligonucleotide probes were used to genotype for HLA-DRB1, which indicated that 7% of pairs were HLA-DRB1 mismatched. Approval was obtained from the University of Minnesota institutional review board, and informed consent was provided by all individuals according to the Declaration of Helsinki.

Retrospective cytokine and HLA typing

Genotyping was performed on stored DNA or crude cell lysate. The quantity of DNA did not allow all pairs to be analyzed at all loci. Cytokine SNPs and HLA class I alleles were genotyped using induced heteroduplex generator (IHG) technology.[18] Analysis of the *TNFSF2* region and *IL10* microsatellites was performed using standard polymerase chain reaction (PCR) methodology.

Statistical analysis

For *IL10* haplotype analysis, 2 previously described methods were used to produce a physically linked 4-locus haplotype (10G, −1082, −819, −592).[12,19] A PHASE-based statistical approach was used to generate haplotypes including the IL-10R microsatellite.[20] For the *TNFSF2* region, the PHASE-based approach was used alone because of large distances between individual polymorphic loci. Cumulative incidence rates and their 95% confidence intervals (CIs) were used to estimate treatment-related mortality, treating relapse-related deaths as a competing risk.[21] Univariate comparisons of the major end points were completed by using the log-rank statistic. In preparation for inclusion into the multiple regression models, the proportional hazards assumption was tested by the Cox regression model using the Wald chi-square test and a time-dependent covariate of the logarithm of time. Cox proportional hazard models were then used to evaluate the independent effect of cytokine polymorphism.[22] In addition to all significant *TNFSF2* region and *IL10* genotype factors, other potential confounders were included in the Cox regression models. These factors included recipient age, donor age, sex, sex mismatch, diagnosis, disease status, T-cell depletion, pretransplantation conditioning, and HLA disparity.

Results and discussion

Relevant allele frequencies for both recipients and donors are summarized in Table 1. No significant differences were found between allele frequencies for *TNFSF2* region and *IL10* in the recipient and donor cohorts. In contrast to ID-SCT studies there was a considerable level of mismatch of *TNFSF2* region genotypes between recipients and donors. At the *TNFSF2* −238 locus, 8.8% of pairs were mismatched for one or more alleles and at the *TNFSF2* −308 locus 13.3% of pairs were mismatched. As the *TNFSF2* region is in linkage disequilibrium with flanking HLA genes, this level of mismatch is consistent with the levels of HLA allele disparity found using the IHG-based HLA typing technique.[18]

Table 1. Cytokine genotypes and haplotypes associated with increased or decreased risk of TRM in unrelated donor SCT at 12 months

Locus/haplotype and allele(s)	Allele frequency*	Status	Phenotype	No. died/total	CI of TRM at 12 months, % (95% CI, %)	*P*	CI of relapse at 12 months, % (95% CI, %)
Overall	NA	NA	NA	73/182	40 (33-47)	NA	12 (7-17)
TNFSF2–1031C	0.2	R	C+	25/46	54 (38-70)	.04	5 (0-11)
TNFSF2–1031C	0.2	R	C−	36/111	32 (23-41)		13 (7-19)
TNF*d4*	0.22	R	d4+	35/62	56 (42-70)	< .01	3 (0-7)
TNF*d4*	0.22	R	d4−	30/99	30 (21-39)		14 (7-21)
TNF*d4*	0.22	D	d4+	34/65	52 (39-65)	.02	11 (4-18)
TNF*d4*	0.22	D	d4−	33/99	33 (24-42)		11 (5-17)
TNF*a5*	0.06	R	a5+	12/17	71 (45-97)	< .01	12 (0-26)
TNF*a5*	0.06	R	a5−	57/154	37 (29-45)		12 (7-17)
TNF*a6*	0.16	D	a6+	15/50	30 (17-43)	.05	20 (9-31)
TNF*a6*	0.16	D	a6−	54/118	46 (37-55)		8 (3-13)
TNF*a7*	0.05	D	a7+	12/18	67 (42-92)	< .01	0
TNF*a7*	0.05	D	a7−	57/150	38 (30-46)		13 (8-18)
TNF*d4*/*TNFSF2*–1031C	NA	R or D	d4+/C+	42/77	55 (43-67)	< .01	9 (3-15)
TNF*d4*/*TNFSF2*–1031C	NA	R or D	Others	14/68	21 (12-30)		12 (8-16)
IL10 R2-G-C-C	NA	D	R2-G-C-C+	22/36	61 (43-79)	.01	11 (1-21)
IL10 R2-G-C-C	NA	D	Others	33/97	34 (25-43)		10 (4-16)
IL10 R3-G-C-C	NA	D	R3-G-C-C+	21/69	30 (19-41)	.01	13 (5-21)
IL10 R3-G-C-C	NA	D	Others	34/64	53 (40-66)		8 (2-14)

Univariate comparisons were made to analyze associations between TNFSF2 region and IL10 polymorphisms previously implicated as predictive of TRM. Only univariate statistically significant associations are shown in the Table. Factors that remained statistically significant in the Cox regression model included TNFd4/TNFSF2 -1031C and TNFa5.

NA indicates not applicable; R, recipient; and D, donor.

*Allele frequencies were all in Hardy-Weinberg equilibrium. IL10 haplotypes were determined by physical linkage.[19]

The association between donor and recipient *TNFSF2* region and *IL10* genotypes and the cumulative incidence of TRM at one year was investigated (Table 1). The presence of the TNF*d4* allele in conjunction with the *TNFSF2* −1031C allele in recipients or donors significantly increased the cumulative incidence of TRM ($P < .01$; Figure 1A). Cox proportional hazards analysis showed that the TNF*d4*/*TNFSF2* −1031 haplotype increased the risk of TRM at one year independent of all other factors. Relative risk (RR) = 2.2, CI 1.2-4.3, $P = .02$; and TNF*a5* RR = 2.8, CI 1.2-6.2, $P = .01$. The other *TNFSF2* region genotypes investigated were not associated with TRM.

Individual *IL10* polymorphic loci were not associated with TRM. However haplotype analysis revealed significant associations. The cumulative incidence of TRM at one year was higher in the presence of R2-G-C-C haplotype[10] ($P = .01$; Figure 1B), whereas the R3-G-C-C haplotype was shown to be protective ($P = .01$; Figure 1C).

Cox proportional hazards analysis supported the association of the R3-G-C-C haplotype with TRM (RR = 0.4, CI 0.2-0.8, $P = .03$), although results could not be verified for the R2-G-C-C haplotype as hazards were not proportional. Cavet et al[12] reported that ID-SC transplant recipients possessing longer 10G alleles were more likely to develop grades II to IV aGVHD. We have shown R2-G-C-C, a haplotype with predominantly long 10G alleles, is a risk factor for TRM after UD-SCT. The patients included in this study were a heterogeneous group. Furthermore, it must be emphasized that these analyses involved multiple comparisons and therefore *P* values between .05 and .01 should be interpreted with caution.

This is the first study to describe an association of cytokine polymorphism with TRM after UD-SCT, although no independent associations with aGVHD were identified. The most important finding is that recipient or donor TNF*d4*/*TNFSF2* −1031C haplotype is a risk factor for TRM (Table 1). The same TNF*d4* allele has recently been implicated as a risk factor for moderate to severe aGVHD in ID-SCT.[16] It is likely that the TNF*d4* allele is part of an extended chromosome 6 haplotype comprising immune HLA and a complement gene. We have shown significant associations between polymorphisms of *TNFSF2* region and *IL10* with TRM after UD-SCT. Confirmation of our results in a multicenter context would rule out any possibility of a center effect being responsible for our findings. Cytokine gene polymorphism analysis could become a useful pretransplantation tool to identify high-risk donors and recipients.

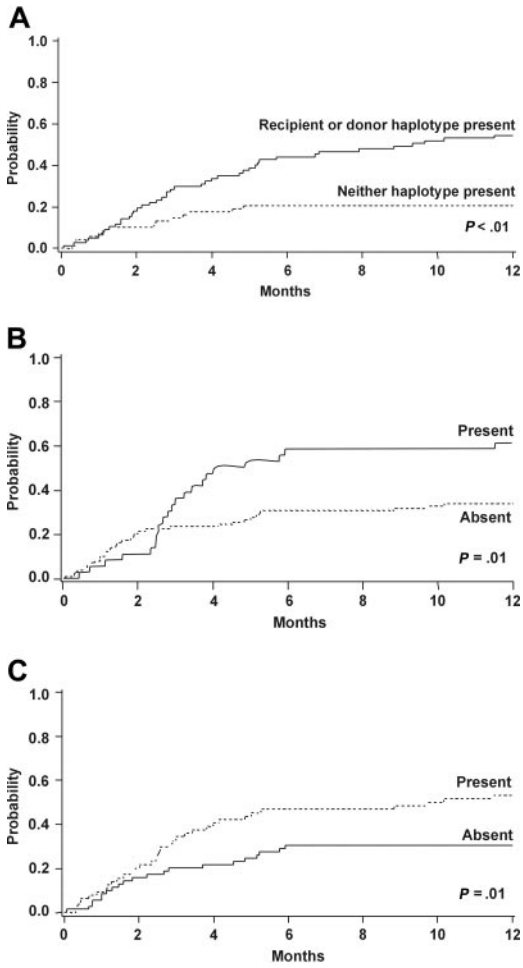

Figure 1. Cumulative incidence plots. (A) Cumulative incidence plot showing associations between transplant-related mortality (TRM) and presence or absence of the TNFd4+, 1031(C+) haplotype in donors and recipients of unrelated donor stem cell transplants. The cumulative incidence of TRM at one year after transplantation when the donor or recipient were TNFd4+, 1031(C+) haplotype–positive was 55% (43%-67%) compared with 21% (12%-30%) when both were negative ($P < .01$). (B) Cumulative incidence plot showing associations between transplant-related mortality and donor R2-G-C-C haplotype. The cumulative incidence of TRM at one year was significantly higher when the R2-G-C-C haplotype was present in the donor (61% [43%-79%]) compared with when it was absent from the donor (34% [25%-43%]), $P = .01$. (C) Cumulative incidence plot showing associations between transplant-related mortality and donor R3-G-C-C haplotype. The cumulative incidence of TRM at one year after transplantation was significantly lower when the R3-G-C-C haplotype was present in the donor (30% [19%-41%]) compared with when the haplotype was absent from the donor (53% [40%-66%]), $P = .01$.

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