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 TNFαd4 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, TNFα5, 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)

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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 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 HLA-DP, DRB1, DQ, and 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. 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). A PHASE-based statistical approach was used to generate haplotypes including the IL-10R microsatellite. 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. 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 log of time. Cox proportional hazard models were then used to evaluate the independent effect of cytokine polymorphism. 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.
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\textdagger symbol \textdagger 4 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 TNFD\textdagger symbol \textdagger 4/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\textdagger symbol \textdagger 6 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 haplotypic analysis revealed significant associations. The cumulative incidence of TRM at one year was higher in the presence of R2-G-C-C haplotype\textsuperscript{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\textsuperscript{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 TNFD\textdagger symbol \textdagger 4/TNFSF2 – 1031C haplotype is a risk factor for TRM (Table 1). The same TNFD\textdagger symbol \textdagger 4 allele has recently been implicated as a risk factor for severe aGVHD in ID-SCT.\textsuperscript{16} It is likely that the TNFD\textdagger symbol \textdagger 4 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.

References

12. Cavet J, Middleton PG, Segall M, Noreen H, Daves SM, Dickinson AM. Recipient tumor necrosis...


