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

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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)

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

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Financial disclosure: See Acknowledgments on page 549.

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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