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Frequency and Targeted Detection of HLA-DPB1 T Cell Epitope Disparities Relevant in Unrelated Hematopoietic Stem Cell Transplantation

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ABSTRACT

The majority of unrelated donor (UD) hematopoietic stem cell (HSC) transplants are performed across HLA-DP mismatches, which, if involving disparity in a host-versus-graft (HVG) direction for an alloreactive T cell epitope (TCE), have been shown by our group to be associated with poor clinical outcome in 2 cohorts of patients transplanted for hematopoietic malignancies and beta-thalassemia, respectively. Using site-directed mutagenesis of DPB1*0901, we show here that the TCE is abrogated by the presence of amino acids LFQG in positions 8-11 of the DP beta-chain. Based on this and on alloreactive T cell responsiveness, we have determined the presence or absence of the TCE for 72 DPB1 alleles reported in the ethnic groups representative of the worldwide UD registries, and predict that 67%-87% (mean 77%) of UD recipient pairs will not present a DPB1 TCE disparity in the HVG direction. We developed and validated in 112 healthy Italian blood donors an innovative approach of DPB1 epitope-specific typing (EST), based on 2 PCR reactions. Our data show that DPB1 TCE disparities may hamper the clinical success of a considerable number of transplants when DPB1 matching is not included into the donor selection criteria, and that a donor without DPB1 TCE disparities in the HVG direction can be found for the majority of patients. Moreover, the study describes the first protocol of targeted epitope-specific DPB1 donor-recipient matching for unrelated HSC transplantation. This protocol will facilitate large-scale retrospective clinical studies warranted to more precisely determine the clinical relevance of DPB1 TCE disparities in different transplant conditions.

KEY WORDS

HLA-DPB1 • T cell epitopes • Epitope-specific typing • Unrelated hematopoietic stem cell transplantation • Allelic frequencies

INTRODUCTION

Hematopoietic stem cell (HSC) transplantation from matched unrelated donors (UDs) is increasingly being used both in the United States (CIBMTR, Center for International Blood and Marrow Transplants Research; http://www.ibmtr.org) and in Europe [1] for the cure of a variety of neoplastic or genetic diseases of the hematopoietic system, including leukemia, lymphoma, multiple myeloma, or severe combined immunodeficiency (SCID) and beta-thalassemia [2,3]. The clinical success of unrelated HSC transplantation is critically dependent on the degree of UD-recipient matching for the human leukocyte antigen (HLA) class I and II antigens (Ags) encoded within the major histocompatibility complex (MHC). Despite an increasingly growing number of HLA-typed volunteers enrolled in the world-wide registries, which to date exceeds 11 million, the probability of

finding a UD minimally matched for low-resolution HLA-A, B, and high-resolution HLA-DRB1 alleles (6/6 alleles) varies from 80% in Whites to <40% in ethnic minorities (NMDP, National Marrow Donor Program; http://www.marrow.org) [4]. This probability decreases to <40% when trying to identify a UD matched for the additional loci HLA-C and DQB1 (10/10 alleles) [5], and is <20% if identity for HLA-DPB1 is included into the matching criteria (12/12 alleles) [6].

The current recommendations of the American NMDP include high-resolution UD-recipient matching for HLA-A, B, C, and DRB1, whereas disparity for DQB1 and DPB1 is not considered to be relevant [7-9]. Because of weak linkage disequilibrium between DPB1 and the other HLA class II loci [10-12], over 80% of UD transplants are performed across mismatches at DPB1 [6]. On the other hand, numerous studies including our own have shown in the past that the degree of DPB1 matching can have an impact on the outcome of HSC transplantation from UDs [6,13-18]. In particular, we and others have shown that HLA-DP Ags can be targets of acute immune-mediated HSC allograft rejection [19] and acute graft-versus-host-disease (aGVHD) [20,21]. On the basis of reactivity by HLA-DP-specific alloreactive T cells we have recently developed an algorithm for UD-recipient disparities for a shared T cell epitope (TCE) encoded by a subset of DPB1 alleles [13]. According to this algorithm, individuals carrying self-DPB1 alleles encoding the TCE will have clonally deleted alloreactive T cells for the shared epitope, whereas individuals carrying self-DPB1 alleles lacking the TCE will have retained TCE-specific alloreactive T cells in their repertoire. Depending on the combination of DPB1 alleles present in the donor and in the recipient of an unrelated stem cell transplantation (SCT), DPB1 disparities can therefore be classified as TCE permissive (both donor and recipient or none of the 2 carry at least 1 DPB1 allele encoding the TCE) or TCE disparate. TCE disparities can be either in the HVG direction (nonpermissive HVG: the donor carries at least 1 DPB1 allele encoding the TCE, whereas the recipient does not), or in the GVH direction (nonpermissive GVH: the recipient carries at least 1 DPB1 allele encoding the TCE whereas the donor does not). According to these definitions, TCE matching status for a donor-recipient pair is unambiguous, and disparities in HVG and GVH directions are mutually exclusive. In our retrospective clinical studies, DPB1 TCE disparities were found to be associated with an increased risk of aGVHD and treatment-related mortality (TRM) in oncohematologic patients [13] and of graft rejection in beta-thalassemia patients [22].

In the present study, we have used site-directed mutagenesis to define the presence or absence of the DPB1 TCE in 72 out of 114 DPB1 alleles reported to date, and calculated the predicted frequency of DPB1 TCE donor-recipient disparities in various ethnic groups. Moreover, we present an innovative protocol for rapid, targeted detection of DPB1 TCE disparities by epitope-specific typing (EST), which will improve the feasibility of large-scale retrospective clinical studies needed to better define the clinical relevance of DPB1 TCE disparities in different transplant settings.

MATERIALS AND METHODS

References Cell Lines

Epstein Barr Virus (EBV) transformed B lymphoblastoid cell lines (BLCL) from the Xth International Histocompatibility Workshop, available through the European Collection of Animal Cell Cultures, were used as controls for both conventional allele-specific DPB1 typing and EST. DPB1 typing of these BLCLs was as follows: V.E.C. (DPB1*0401, *4601), WT100BIS (DPB1*0101), H0301 (DPB1*0501), BEL-8-CC (DPB1*0202, *2001), KT14 (DPB1*0201, *1901), CML (DPB1*1401, *0401), and BM21 (DPB1*1001). The following BLCLs were established from peripheral blood mononuclear cells (PBMCs) from donors DPB1 typed in our laboratory: HSR-DAV (patient BLCL from our previous study [13], DPB1*0201, *0401), HSR-NAM (DPB1*0201, *0502), HSR-190 (DPB1*0402, *3401), HSR-FOCS (DPB1*0401, *3501), HSR-MB (DPB1*0901, *1101), HSR-SL (DPB1*0201, *1701), HSR-366 (DPB1*0401, *1401), and HSR-0208 (DPB1*0402, *1501).

Site-Directed Mutagenesis of DPB1*0901 and Expression of Mutant Molecules

We generated 2 site-directed mutants of DPB1*0901, designated DPB1*09LF and DPB1*09M/GGPM, encoding aa LFQG in positions 8-11 and M/GGPM in positions 76/84-87 of the DP beta-chain, respectively (Figure 1). DPB1*09M/GGPM is identical in sequence to the naturally occurring DPB1*8601 (The American Society for Histocompatibility and Immunogenetics; http://www.ashi-hla.org) [23,24]. The mutants were generated starting from 2 retroviral vector constructs carrying the full-length DPB1 cDNA, LDPB1*0901SΔN and LDPB1*4601SΔN, described previously [13]. Two 643-bp fragments containing the 214 3′ codons of the DPB1*0901 or DPB1*4601 coding regions were removed from the respective vectors by restriction endonucleases cleavage using an internal 5′ *Mlu* I site (Figure 1) and a 3′ *Xho*I site in the vector's multiple cloning region, and mutually swapped. Plasmids encoding mutant molecules were cloned and the full-length 777-bp DPB1 coding region was sequenced on both strands.

Mutant molecules were expressed in the HSR-DAV BLCL by retroviral vector-mediated gene transfer as described, and transduced cells were purified by