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Effects of transmembrane region variability on cell surface expression and allorecognition of HLA-DP3

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ABSTRACT

The functional relevance of polymorphisms outside the peptide binding groove of HLA molecules is poorly understood. Here we have addressed this issue by studying HLA-DP3, a common antigen relevant for functional matching algorithms of unrelated hematopoietic stem cell transplantation (HSCT) encoded by two transmembrane (TM) region variants, DPB1*03:01 and DPB1*104:01. The two HLA-DP3 variants were found at a overall allelic frequency of 10.4% in 201 volunteer stem cell donors, at a ratio of 4.2:1. No significant differences were observed in cell surface expression levels of the two variants on B lymphoblastoid cell lines (BLCL), primary B cells or monocytes. Three different alloreactive T cell lines or clones showed similar levels of activation marker CD107a and/or CD137 upregulation in response to HLA-DP3 encoded by DPB1*03:01 and DPB1*104:01, either endogenously on BLCL or after lentiveral-vector mediated transfer into the same cellular background. These data provide, for the first time, direct evidence for a limited functional role of a TM region polymorphism on expression and allorecognition of HLA-DP3 and are compatible with the notion that the two variants can be considered as a single functional entity for unrelated stem cell donor selection.

1. Introduction

It is well established that the functionally most important part of the HLA molecule, in terms of antigen presentation and T cell recognition, is the peptide antigen binding groove, which is encoded by polymorphic exons 2 and 3 for HLA class I and exon 2 for HLA class II [1,2]. In line with this notion, numerous studies have found an association between the clinical outcome of volunteer unrelated donor (VUD)-hematopoietic stem cell transplantation (HSCT) and disparity for mismatches inside the antigen binding groove [3–6]. Based on these findings, the internationally accepted definitions of histocompatibility typing terms limit the requirements for high resolution typing in VUD searches for HSCT to expressed alleles that encode the same protein sequence for the antigen binding groove [7]. As a consequence, for the majority of newly reported HLA alleles, the outside-the-groove sequence is unknown. For instance, nucleotide sequences beyond exon 2 are available only for 53/155 of the known HLA-DPB1 alleles to date (<www.ebi.ac.uk/imgt/hla>) [8–10]. Likewise, limited data are available regarding the functional or clinical impact of outside-the-groove variability. It has been shown that some of these polymorphisms can influence HLA surface expression by transcriptional regulation, alternative splicing or structural changes of the molecule [11–13], while others did not change the repertoire of bound peptides [14]. From the clinical side, a polymorphism in exon 3 of DRB1*14:01/14:54 was shown not to have a significant impact on the outcome of unrelated VUD-HSCT [15,16]. None of

Abbreviations: HSCT, hematopoietic stem cell transplantation; VUD, volunteer unrelated donors; GvHD, graft versus host disease; TM, transmembrane; PCR-SSP, PCR-sequence specific priming; BLCL, Epstein–Barr Virus transformed B lymphoblastoid cell lines; MLR, mixed lymphocyte reaction; R, responder donor; S, stimulator donor; PBMC, peripheral blood mononuclear cells; MFI, median fluorescence intensity; MESF, molecules of equivalent soluble fluorochrome; γ-IFN, gamma interferon; LV, lentiviral expression vector; ΔLNGFR, truncated form of the human low affinity nerve growth factor receptor; hPGK, human phosphoglycerate kinase; TCE, T cell epitope.

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the to date available studies have directly addressed the effects of outside-the-groove polymorphisms on T cell alloreactivity, the most relevant factor for graft versus host disease (GvHD), graft versus tumor activity and engraftment after allogeneic HSCT.

Here we have addressed this question for HLA-DP3, an antigen present with a phenotypic frequency of about 20% in the Caucasian population [17,18], which has been shown to be relevant in functional matching algorithms associated with mortality, GvHD and oncohematologic disease relapse after VUD-HSCT [19–21]. The HLA-DP3 beta-chain is encoded by two variants, DPB1*03:01 and DPB1*104:01, differing only for an amino acid substitution from valine to methionine at position 205 of the transmembrane (TM) region [8,9]. We set out to determine the relative frequency of the two variants and to better understand their functional implication in cell surface antigen expression and T cell alloreactivity.

2. Materials and methods

2.1. HLA-DPB1 typing

Genomic high resolution HLA-DPB1 typing was performed by PCR-Sequence Specific Priming (PCR-SSP) using the Olerup™ kit (Olerup GmbH, Vienna, Austria), according to the manufacturer's recommendations.

2.2. Study population

HLA-DPB1 allele frequencies were determined from 201 adult volunteer unrelated donors (VUD), mostly from Italian, German and American Registries, each selected for a different onco-hematologic patient, mainly of Caucasian origin, referring to the San Raffaele Scientific Institute in Milan for a stem cell donor search under institutionally approved protocols.

2.3. B lymphoblastoid cell lines

HLA-typed Epstein–Barr Virus transformed B lymphoblastoid cell lines (BLCL) were established according to standard protocols or purchased from the European Collection of Animal Cell Cultures (ECACC).

2.4. Monoclonal antibodies

The following monoclonal antibodies (mAb) were used: L243 (unconjugated anti-HLA-DR, Biolegend, Uithoorn, The Netherlands; or phycoerythrin (PE)-conjugated anti-HLA-DR, Becton Dickinson (BD), Milan, Italy); B7/21 (unconjugated anti-HLA-DP, kindly provided by Sir Walter Bodmer, London, UK; or PE-conjugated anti-HLA-DP, Abcam, Cambridge, UK); TL-3B6 (unconjugated anti-HLA-DP-DEAV$_{(84–87)}$, locally produced); W6/32 (unconjugated anti-HLA class I, Biolegend); ME20.4 (allophycocyanin (APC)-conjugated anti-nerve growth factor receptor (NGFR), Miltenyi Biotec Bergisch Gladbach, Germany); SK7 (conjugated anti-CD3-PE-Cy7, BD); MφP9 (conjugated anti-CD14-fluorescein isothiocyanate (FITC), BD); HIB19 (conjugated anti-CD19-APC, BD); RPA-T4 (conjugated anti-CD4-APC, BD); 4B4-1 (conjugated anti-CD137-PE, BD); H4A3 (conjugated anti-CD107a-FITC, BD).

2.5. Isolation of alloreactive T cell effectors

T cells alloreactive to HLA-DP3 encoded by DPB1*03:01 were obtained by classical 1-way Mixed Lymphocyte Reactions (MLRs) between responder (R) and stimulator (S) peripheral blood mononuclear cells (PBMC) from VUD pairs matched for 8-10/10 of the HLA-A, -B, -C, -DRB1 and -DQB1 alleles. Briefly, R-PBMC were stimulated twice with irradiated S-PBMC at a 1:1 ratio in the presence of 150 UI/ml interleukin-2 (IL-2; Novartis, Varese, Italy). Alloreactive effector cells were maintained before (M3-VR/FV-3) or after (C2-DK/DK-3 and C11-DK/MN-3) limiting dilution cloning in the presence of 300 UI/ml IL-2, irradiated S-BLCL and third party feeder PBMC according to previously reported methods [22].

2.6. T cell activation marker upregulation assays

Functional analysis of alloreactive effector T cells was performed by flowcytometric quantification of activation markers CD137 or CD107a [23,24]. For CD137 upregulation, T cells were incubated with target BLCL at a 1.5:1 ratio for 24 h and stained with appropriately labeled anti-CD4 and anti-CD137 mAb. For analysis of CD107a upregulation, T cells were incubated with target BLCL at a 1.5:1 ratio for 1 h in the presence of labeled anti-CD107a mAb, followed by 3 h incubation with monensin-A (Sigma–Aldrich, Milan, Italy) and staining with appropriately labeled mAb anti-CD4. All T cell effectors were initially tested against DPB1*03:01 by both CD137 and CD107a assays, and the most informative assay, i.e. the assay using the marker with highest specific up-regulation after alloantigen encounter for each T cell, was selected for further analysis. For mAb inhibition studies, target BLCL were pre-incubated with the relevant unconjugated mAb at a final concentration of 30 μg/ml, before addition of T cell effectors. For target-titration experiments, a fixed number of alloreactive T cells (5×10^4) was stimulated with 2-fold serial dilutions of target BLCL, starting from an effector:target ratio of 1:1. Results were represented as relative percentage of activated T cells calculated with the following formula: (% activated cells$_{[\text{target dilution}]}$ − baseline$_{[\text{Negative control}]}$)/(% activated cells$_{[\text{undiluted target}]}$ − baseline$_{[\text{Negative control}]}$).

2.7. Quantitative assessment of cell surface antigen expression

Quantitative evaluation of surface antigen expression was performed by converting Median Fluorescence Intensity (MFI) Values into the respective Molecules of Equivalent Soluble Fluorochrome (MESF) [25], using linear regression analysis of a standard curve generated using SPHERO Rainbow Calibration Particles (Spherotech, Lake Forest, USA) according to the manufacturer's instructions. MESF values were corrected subtracting the MESF value of background fluorescence in unstained cells or in cells stained with secondary antibody alone. Cell staining was performed as follows: BLCL were directly stained with the relevant anti-HLA mAb; PBMC from healthy donors were cultured in the presence or absence of 200 UI/ml gamma interferon (γ-IFN) for 48 h, then quantitative evaluation of HLA expression was performed on CD3−/CD14+ or CD3−/CD19+ cells after exclusion of dead cells by staining with Live/Dead Fixable Violet Dead Cell Stain Kit (Life Technologies, Monza, Italy).

2.8. Lentiviral vector mediated gene transfer of DPB1 into BLCL

The 6.9 Kb full-length cDNA of HLA-DPB1*03:01, DPB1*104:01 and DPB1*01:01 was amplified by RT-PCR from HLA typed BLCL using primers previously described [22], sequenced and subcloned into a bidirectional promoter lentiviral (LV) expression vector, kindly provided by Professor Luigi Naldini, Milan, Italy [26]. DPB1 and the truncated form of the human low affinity nerve growth factor receptor (ΔLNGFR) were under the control of the human phosphoglycerate kinase (hPGK) promoter and a minimal core promoter derived from cytomegalovirus fused to the hPGK promoter in the opposite direction, respectively. Target cells were infected by standard protocols [26], and purified by magnetic bead selection (Myltenyi Biotec) for the ΔLNGFR reporter.