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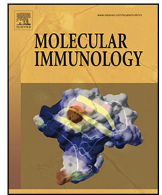

Allorecognition of HLA-DP by CD4+ T cells is affected by polymorphism in its alpha chain

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ABSTRACT

Alloreactivity to HLA-DP molecules, class II heterodimers of an oligomorphic alpha and a polymorphic beta chain, is increasingly being studied due to its relevance in clinical transplantation. We hypothesized that not only polymorphisms in the peptide binding groove encoded by exon 2 of HLA-DPB1, but also in other regions of the molecule and the alpha chain, could play a role in CD4+ T cell allorecognition. To test this possibility, we comparatively investigated CD4+ T cell allorecognition, measured by upregulation of the activation marker CD137, against HLA-DPB1*13:01, *05:01, *03:01, *17:01 or their allele counter parts DPB1*107:01, *135:01, *104:01, *131:01, with identical exon 2 sequences but polymorphism in exons 1, 3 or 4, in the context of different HLA-DPA1 (DPA1) polymorphisms (DPA1*01:03 and *02:01). No significant differences in CD4+ T cell allorecognition levels could be demonstrated for any of the beyond exon 2 DPB1 variants studied. Interestingly, however, the mean fold change in CD4+ CD137+ cells was significantly higher when the target shared at least one DPA1 allele with the allogeneic stimulator, compared to a distinct DPA1 background (1.65 vs 0.23, $P < 0.005$). Structural homology modeling suggested specific amino acid residues in the alpha chain, in particular position 31, to impact CD4+ T cell allorecognition of HLA-DP. Our data argue against a significant role of beyond exon 2 DPB1 polymorphisms for T cell alloreactivity, but show relevance of DPA1 polymorphism in this mechanism. These new findings impact HLA matching strategies in unrelated stem cell transplantation.

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1. Introduction

HLA-DP represents one of the HLA class II molecules, that binds peptides derived from extracellular proteins and presents them on the surface of antigen presenting cells to CD4+ T cells. HLA-DP is expressed on the cell surface as a heterodimer consisting of an alpha chain encoded by the oligomorphic DPA1 gene and a beta chain encoded by the highly polymorphic DPB1 gene (Cresswell, 1994). HLA-DP has been shown to play an important functional role in several immune-related conditions including autoimmunity (Field et al., 2010; Varney et al., 2010), beryllium disease (Richeldi et al., 1993) and tumor and viral antigen recognition (Dong et al., 1995; Castelli et al., 2002; Voo et al., 2002; Landais et al., 2006; Meng et al., 2011; Straetemans et al., 2012). Accumulating evidence over recent years has also shown that alloreactivity to HLA-DP plays an important role in transplantation, both solid organs (Goral et al., 2008; Thaunat et al., 2009; Billen et al., 2010; Jolly et al., 2012) and hematopoietic stem cells (HSCT). In the latter setting, T cell alloreactivity against HLA-DP mismatches expressed by the patient cells has been shown to be associated with a higher risk to graft-versus-host-disease (GVHD) and disease relapse (Varney et al., 1999; Petersdorf et al., 2001; Shaw et al., 2007). In contrast, HLA-DP mismatching was not associated with GVHD or survival in various other studies (Flomenberg et al., 2004; Lee et al., 2007; Eapen et al., 2010). More recently, functional donor–recipient matching for alloreactive T cell epitope groups has been shown to identify permissive or non-permissive mismatches associated with clinical risks after unrelated HSCT (Zino et al., 2004; Crocchiolo et al., 2009; Fleischhauer et al., 2012).

Based on these results, interest in the mechanisms underlying T cell alloreactivity to HLA-DP is rising. Most functional studies

Abbreviations: HSCT, hematopoietic stem cell transplantation; GVHD, graft-versus-host-disease; DPB1, HLA-DPB1 gene; DPA1, HLA-DPA1 gene; LD, linkage disequilibrium.

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Table 1
HLA-DPB1, -DPA1, -DRB1 and -DQB1 typing of BLCLs used in this study.

BLCL[a]	DPB1*	DPA1*	DRB1*	DQB1*
MGAR	04:01	01:03	15:01	06:02
MGAR03[a]	04:01, **03:01*[b]	01:03	15:01	06:02
MGAR104[a]	04:01, **104:01*[b]	01:03	15:01	06:02
MGAR17	04:01, **17:01*[b]	01:03	15:01	06:02
MGAR131	04:01, **131:01*[b]	01:03	15:01	06:02
VAVY	01:01	02:01	03:01	02:01
VAVY03	01:01, **03:01*[b]	02:01	03:01	02:01
VAVY104	01:01, **104:01*[b]	02:01	03:01	02:01
VAVY17	01:01, **17:01*[b]	02:01	03:01	02:01
VAVY131	01:01, **131:01*[b]	02:01	03:01	02:01
15745DP13	13:01	02:01	01:01	05:01
15748DP107	107:01	04:01	09:01	03:03
15716DP05	05:01	02:02	14:05, 15:01	05:03, 06:01
30400DP135	135:01	02:02	11:05, 15:02	05:02, 06:02

[a] Transduced BLCL described in Ref. Crivello et al. (2013).
[b] Allele in italic was introduced by LV-mediated transduction.

focused on exon 2 HLA-DPB1 (DPB1) gene polymorphism, encoding the peptide binding groove of the beta chain, as this is the region that is considered to play a dominant role in T cell alloreactivity. A number of groups tried to identify immunogenic epitopes in the peptide binding groove of the beta chain to unravel HLA-DP immunogenicity (Cesbron et al., 1993; Nicholson et al., 1997; Diaz et al., 2003). These studies describe a crucial role for various amino acid residues in the peptide binding groove in T cell allorecognition of the DP molecule, nevertheless T cell alloreactivity to HLA-DP cannot be pointed to these residues.

Knowledge about the functional relevance of polymorphism beyond DPB1 exon 2 is scarce. We previously described, in addition to the known DPB1 polymorphism in exon 2, polymorphism in the exons 1, 3, 4 and 5 (Lauterbach et al., 2012). However, it is currently unknown if these polymorphisms influence T cell allorecognition.

The T cell receptor binds to both the beta- and alpha chain of the HLA-DP molecule in a peptide dependent manner (Hennecke and Wiley, 2002; Kovalik et al., 2000). The HLA-DP alpha chain shows limited polymorphism compared to the beta chain, but still the HLA-DPA1 (DPA1) alleles encode 19 different cell surface proteins (Robinson et al., 2013). Since DPA1 is in strong linkage disequilibrium (LD) with DPB1 (Begovich et al., 2001; Hollenbach et al., 2012), DPA1 is rarely considered in functional or clinical studies of HLA-DP. Gaston et al. were the first to describe an influence of polymorphism in the HLA-DP alpha chain on recognition of a heat shock protein-derived peptide by HLA-DP restricted T cell clones and suggested an important role for the DPA1 amino acid at position 31 in peptide binding affinity (Gaston et al., 1997). Also Reche and Reinherz indicate a role for polymorphic residues of HLA-DPA1 in TCR and peptide binding (Reche and Reinherz, 2003). In HLA-matched unrelated HSCT, donor–recipient DPA1 but not DPB1 disparity has been suggested to be associated with reduced survival and shorter relapse-free survival in a single center study (Schaffer et al., 2003), although this was not confirmed by others (Varney et al., 1999; Flomenberg et al., 2004; Lee et al., 2007). These findings suggest that the role of the HLA-DP alpha chain on T cell allorecognition needs further attention for a better understanding of HLA-DP immunogenicity in transplantation.

In the present study, we hypothesized that allorecognition of HLA-DP by CD4+ T cells could be shaped by polymorphism in the regions beyond the peptide binding groove of the beta chain, and/or by polymorphism in the alpha chain. The study addresses two questions: First, do targets with DPB1 polymorphism in exons 1, 3 or 4 but identical exon 2 sequences have an impact on HLA-DP allorecognition by CD4+ T cells and second, does polymorphism in the alpha chain influence CD4+ T cell allorecognition of HLA-DP? In order to address these questions, we undertook a functional analysis of T cells alloreactive to HLA-DP antigens formed by beta-alpha chain heterodimers encoded by DPB1 alleles with beyond exon 2 variability and the two most frequent DPA1 allele variants, DPA1*01:03 and DPA1*02:01, respectively.

2. Materials and methods

2.1. Cells and HLA-DP typing

Epstein–Bar virus transformed B-cell lymphoblastoid cell lines (BLCLs) were either purchased from the European Collection for Animal Cell Cultures (ECACC) or locally established. Responder (R) and Stimulator (S) cells for mixed lymphocyte reactions (MLR) were obtained from healthy individuals selected as potential donors for unrelated hematopoietic stem cell transplantation for patients at the San Raffaele Scientific Institute in Milan. HLA-DPA1 typing was obtained by PCR-Sequence-Specific Oligonucleotide (SSO) using the luminex technology (Voorter et al., 2010). High resolution DPB1 typing of R/S donor pairs was performed by PCR-Sequence Specific Priming (PCR-SSP) using the Olerup™ kit (Olerup GmbH, Vienna, Austria), according to the manufacturer's recommendations. It should be noted that this method allowed discrimination of the outside exon 2 polymorphisms studied in this work. High resolution HLA-DPB1 typing of target BLCLs used for functional testing of outside exon 2 DPB1 variants was performed by sequence-based typing as previously described (Lauterbach et al., 2012). The DPA1 and DPB1 typing results of the cells are shown in Tables 1 and 2.

2.2. Monoclonal antibodies and flow cytometry

Monoclonal antibodies (mAb) anti-HLA-DR (L243 unconjugated, Biolegend, Uithoorn, The Netherlands) and anti-HLA-DP (B7/21 unconjugated, kindly provided by Sir W. Bodmer, London, UK) were used as blocking mAbs in functional assays. The mAb anti-NGFR (ME20.4 unconjugated, Miltenyi Biotec Bergisch Gladbach, Germany) was used to analyze ΔLNGFR expression in transduced cell lines, as the truncated human Low affinity Nerve Growth Factor Receptor was incorporated in the vector as marker to monitor NGFR surface expression and transduction efficiency. The unconjugated human mAb anti-HLA-DP directed against DEAV(84–87) motif (TL-3B6), kindly provided by Dr. Arend Mulder, was used to measure transgenic HLA-DP expression in the transduced cell lines. To detect this human IgG specific for HLA-DP, a FITC-conjugated AffiniPure F(ab′)2 fragment of goat anti-Human IgG antibody specific for the Fc-gamma fragment was used (Jackson ImmunoResearch, West-Grove, USA). The mAbs anti-CD4 (RPA-T4, allophycocyanin (APC)-conjugated, Becton Dickinson (BD), Milan,

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