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Retransplant candidates have donor-specific antibodies that react with structurally defined HLA-DR,DQ,DP epitopes☆

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Abstract

This report describes a detailed analysis how donor-specific HLA class II epitope mismatching affects antibody reactivity patterns in 75 solid organ transplant recipients with an *in situ* allograft and who were considered for retransplantation. Sera were tested for antibodies in a sensitive antigen-binding assay (Luminex) with single class II alleles. Their reactivity was analyzed with HLAMatchmaker, a structural matching algorithm that considers so-called eplets to define epitopes recognized by antibodies. Only 24% of the patients showed donor-specific anti-DRB1 antibodies and there was a significant correlation with a low number of mismatched DRB1 eplets. This low detection rate of anti-DRB1 antibodies may also be due to allograft absorption. In contrast, antibodies to DRB3/4/5 mismatches were more common. Especially, 83% of the DRB4 (DR53) mismatches resulted in detectable antibodies against an eplet uniquely found on DR53 antigens.

Donor-specific DQB mismatches led to detectable anti-DQB antibodies with a frequency of 87%. Their specificity correlated with eplets uniquely found on DQ1-4. The incidence of antibodies induced by 2-digit DQA mismatches was 64% and several eplets appeared to play a dominant role. These findings suggest that both α and β chains of HLA-DQ heterodimers have immunogenic epitopes that can elicit specific antibodies. About one-third of the sera had anti-DP antibodies; they reacted primarily with two DPB eplets and an allelic pair of DPA eplets.

These data demonstrate that HLA class II reactive sera display distinct specificity patterns associated with structurally defined epitopes on different HLA-D alleles.

Keywords: HLA; Epitope; Alloantibody; HLAMatchmaker; Eplet; Allosensitization; Retransplantation; Donor-specific anti-class II antibody

1. Introduction

Humoral immune responses to class II HLA antigens affect the outcome of various types of organ transplants. Preformed anti-donor class II antibodies increase the risk of transplant failure [1–9] and the post-transplant development of anti-class II antibodies is associated with a higher incidence of acute and chronic rejection [10–19].

Current class II matching strategies for kidney transplantation consider only the HLA-DR antigens controlled by the DRB1 locus but mismatching for HLA-DQ and HLA-DP may also lead to lower graft survival rates [20–25]. Newer serum screening methods such as ELISA, Flow Cytometry and Luminex have greatly enhanced the detection of anti-HLA-DQ and HLA-DP antibodies and their association with transplant rejection [2,7,26–29]. Nevertheless, the clinical relevance of these anti-class II antibodies has remained a controversial issue.

Antibodies react with epitopes on antigenic molecules and a characterization of the antibody response to class II epitopes rather than antigens seems important for the management of sensitized patients considered for retransplantation. In this report we address the question whether in the presence of the allograft, circulating antibodies can be detected that are specific for epitopes on donor HLA-DR, HLA-DQ and HLA-DP mismatches. Class II antigens have generally lower levels of tissue expression than class I antigens and this may affect the ability of the allograft to absorb donor-specific anti-class II antibodies. Serum testing for antibodies was done with a highly sensitive antibody-binding assay with

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single allele panels using the Luminex platform [30]. Antibody reactivity patterns were analyzed with HLAMatchmaker, a structural matching algorithm that considers amino acid residue polymorphisms to define epitopes recognized by antibodies. We have applied a recent version that uses so-called eplets defined by molecular surface-exposed polymorphic residues surrounded by residues within a three-Angstrom radius as previously described [31,32]. The data demonstrate distinct antibody specificity patterns associated with eplets on donor class II antigens encoded by the different HLA-D loci.

2. Patients and methods

2.1. Patients

This analysis was done for 75 class II sensitized patients with different types of failed allografts including sixty kidney, four liver, four heart, two lung, two pancreas and three small bowel transplants. All patients had become candidates for retransplantation and their transplants were still present. A second group consisted of 38 class II sensitized patients who did not have a transplant, including 9 patients from whom the allograft had been removed. This study was approved by the Institutional Review Board of the University of Pittsburgh Medical Center.

2.2. Determination of HLA-DR, -DQ and -DP types

HLA typings of patients and donors were done by standard DNA-based methods and considered only alleles reported as most common in the US population [33]. Since the HLAMatchmaker analysis requires high-resolution (4-digit) types, we have typed as many possible subjects at this level for DRB1, 3, 4, 5 and DQB1. In other cases, the HLAMatchmaker program can assign 4-digit types on the basis of most frequent DRB1-DRB3/4/5-DQB1 combinations according to recently published data about HLA class II haplotype frequencies in different populations [34–36]. The same linkage disequilibrium-based approach was used for assigning 4-digit DQA1 types. An analysis of 59 class II typings has shown that at the 2-digit level, 98% of the predicted DQA1 alleles agreed with the actual typing results and there was a 91% concordance at the 4-digit level (data not shown). We conclude that the prediction model to assign DQA1 alleles is highly reliable. A small group of patients ($N=34$) and donors ($N=9$) were DNA-typed for HLA-DPB1 because these patients had shown anti-DP antibodies. No typing was done for DPA.

2.3. Serum reactivity assays

All sera showed anti-class II antibody activity determined by screening with HLA antigen mixtures in Elisa and/or Luminex assays by standard methods. Antibody specificity was determined with Luminex assays using single allele kits supplied by two commercial vendors (One Lambda, Inc., Canoga Park, CA; Tepnel Life Codes Corporation, Stamford, CT). This combination offers two advantages. First is the opportunity to compare the reactivity pattern for each allele shared by each kit. This antibody detection technology is rather new and it is possible that certain allele preparations give aberrant results. Indeed, our experience has shown major discrepancies for one DRB3*0101 preparation which had a contaminating DRB3∗04 allele and one DQB1*0301 preparation had weak reactivity; they were excluded from our analysis. Other preparations showed minor discrepancies such as comparatively low or high reactivity but this did not interfere with our antibody specificity analysis. The second advantage was that one kit had allelic combinations that were not present in the other kit; this applied especially to the DQ and DP preparations. As shown in Table 1, the combined sets had 26 distinct DRB alleles, 33 unique DQA-DQB heterodimers and 27 unique DPA-DPB heterodimers. For many sera, this combination allowed a more precise analysis of antibody specificity than one kit alone.

Table 1
Class II allele distribution in two commercial Luminex kits

Class II gene product	Both kits	Tepnel [a]	One Lambda [b]	Total
Unique DRB allele	20	0	6	26
Unique DQA-DQB heterodimer	2	15	16	33
Unique DPA-DPB heterodimer	9	14	4	27

[a] Tepnel LifeCodes LSA™ Class II Lot 01207.
[b] OneLambda LABScreen™ Lot #004.

2.4. HLAMatchmaker analysis of serum reactivity with class II panels

Different HLAMatchmaker programs can be downloaded from the www.tpis.edu website. We have used a program to analyze serum reactivity patterns with Luminex single class II alleles. Fig. 1 is an example of a reactivity pattern with DQ heterodimers in the Tepnel panel. The patient who typed as DQB1*0501, 0602; DQA1*0101, 0102 had received a kidney transplant from a one-haplotype matched related donor with a mismatched DQB1*301, DQA1*0501 combination. The mismatched DQB eplets are 14AM, 26Y, 45EV, 52PL, 55PPP, 56PPD, 70RT, 84QL2 and 140T2 and DQA eplets are 41GR3, 56RB, 60QF, 64TI4, 69L and 75SL4 (see Footnote[1]). Any of these eplets may have the potential of inducing specific antibodies. This was determined by analyzing the antibody reactivity with the panel. Serum reactions are shown as MFI values and those above two times the average reactivity with self-alleles (in this case 2×497) were considered positive. The panel had 17 DQ heterodimers and Fig. 1 shows for each one which eplets are non-self for this patient. Six heterodimers gave negative reactions; their non-self eplets were considered non-reactive. The negative DQB and DQA alleles were recorded and the computer program then deleted the non-reactive eplets from the donor and panel alleles. The bottom half of Fig. 1 shows the remaining alleles on the reactive alleles. It can be readily seen that DQB1*0301 (DQ7), *0302 (DQ8) and *0303 (DQ9) share 55PPP, an eplet uniquely found on all DQ3 molecules. DQB1*0302 was especially informative because it shared only 55PPP with the immunizing DQB1*0301. Two eplets 45EV (unique for DQ7) and 56PPD (shared between DQ7 and DQ9) are also on reactive alleles but no informative DQB alleles were in the overall panel to rule out antibodies against these eplets. We conclude that DQ7, 8 and 9 are unacceptable mismatches because of anti-55PPP reactive antibodies. No antibody reactivity was seen with other eplets on the immunizing DQB1*0301 namely, 14AM, 26Y, 70RT, 84QL2 and 140T2. These eplets are acceptable mismatches.

This serum had also donor-specific anti-DQA1 reactivity and there were two eplets on reactive alleles, namely 41GR3 (shared by DQA1*04, *05 and *06) and 75SL4 (on DQA1*05). This suggests that DQA1*04, *05 and *06 are unacceptable mismatches for this patient. The remaining DQA eplets 56RB, 60QF, 64TI4 and 69L appear to be acceptable mismatches.

These findings illustrate that the antibody response generally involve a limited repertoire of eplets on the immunizing allele. The characterization of epitope specificity provides a more affirmative and comprehensive assessment of mismatch acceptability.

3. Statistical methods

Differences in serum reactivity patterns and eplet numbers were compared using two-tailed Student *t*-test and Fischer's exact test.

4. Results

4.1. Incidence of HLA-DR, -DQ and -DP antibodies in HLA class II sensitized patients

The initial analysis was done on two groups of HLA class II sensitized patients. The first had antibodies induced during pregnancy, after blood transfusion and/or a previous transplant that had been

[1] Certain eplets show a number at the end of their notation; it indicates that such eplet represents two or more eplets shared by the same antigen or group of antigens. For instance, 84QL2 represents two eplets 84QL and 90ETT; both are on DQ2, DQ3 and DQ4. For a patient with anti-82QL2 antibodies it is unknown whether they react with 84QL and/or 90ETT. We can conclude however that such antibodies react with the 84QL2 eplet shared by DQ2, DQ3 and DQ4 and these antigens should be considered unacceptable mismatches.