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Class II HLA Epitope Matching—A Strategy to Minimize *De Novo* Donor-Specific Antibody Development and Improve Outcomes

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***De novo* donor-specific antibody (*dn*DSA) develops in 15–25% of renal transplant recipients within 5 years of transplantation and is associated with 40% lower graft survival at 10 years. HLA epitope matching is a novel strategy that may minimize *dn*DSA development. HLAMatchmaker software was used to characterize epitope mismatches at 395 potential HLA-DR/DQ/DP conformational epitopes for 286 donor–recipient pairs. Epitope specificities were assigned using single antigen HLA bead analysis and correlated with known monoclonal alloantibody epitope targets. Locus-specific epitope mismatches were more numerous in patients who developed HLA-DR *dn*DSA alone (21.4 vs. 13.2, $p < 0.02$) or HLA-DQ *dn*DSA alone (27.5 vs. 17.3, $p < 0.001$). An optimal threshold for epitope mismatches (10 for HLA-DR, 17 for HLA-DQ) was defined that was associated with minimal development of Class II *dn*DSA. Applying these thresholds, zero and 2.7% of patients developed *dn*DSA against HLA-DR and HLA-DQ, respectively, after a median of 6.9 years. Epitope specificity analysis revealed that 3 HLA-DR and 3 HLA-DQ epitopes were independent multivariate predictors of Class II *dn*DSA. HLA-DR and DQ epitope matching outperforms traditional low-resolution antigen-based matching and has the potential to minimize the risk of *de novo* Class II DSA development, thereby improving long-term graft outcome.**

Keywords: Antibody-mediated rejection, donor-specific antibody, epitope, kidney transplant

Abbreviations: cAMR, chronic antibody-mediated rejection; cPRA, calculated panel reactive antibody; *dn*DSA, *de novo* donor-specific antibody; MFI, mean fluorescence intensity; TerEp, Terasaki epitope

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Introduction

Despite modern crossmatching techniques and potent immunosuppression, immune-mediated injury remains the major cause of renal transplant dysfunction and loss (1). Specifically, rejection associated with *de novo* donor-specific antibody (*dn*DSA), in particular against Class II HLA donor antigens, is associated with a 40% reduction in graft survival at 10 years (2). The risk factors for the development of *dn*DSA include Class II HLA mismatching between donor and recipient, early T cell–mediated rejection, and inadequate immunosuppression resulting from physician minimization of drug dosing or patient nonadherence (2–5). The presence of DSA is associated with progressive microcirculatory inflammation of the glomeruli and peritubular capillaries, transplant glomerulopathy and interstitial fibrosis (''chronic antibody-mediated rejection'' or cAMR), for which there is currently no proven effective treatment (2,6–8).

Recognizing that HLA matching improves graft survival the Organ Procurement and Transplantation Network kidney allocation policy requires mandatory sharing for zero antigen mismatched kidneys in patients with calculated panel reactive antibody (cPRA) $\geq$ 20%, and up to 2 points are assigned for a zero HLA-DR mismatch (http://optn.transplant.hrsa.gov) (9–11). Because of the importance of Class II *dn*DSA in cAMR, it would be logical to further prioritize HLA-DR and HLA-DQ matching for organ allocation (12). However, it is unknown if current HLA typing by low-resolution antigen or high-resolution allele methods allows for adequate risk stratification to minimize *dn*DSA development. An alternative approach would be to replace the current HLA-based method of allocation with a more

refined epitope-based method in which multiple potential immunogenic sites of the HLA molecule are evaluated. In this model, individual HLA epitopes contribute collectively to the overall immunogenicity of the mismatch, with epitope load or specific immunogenic HLA epitopes driving the formation of *dn*DSA posttransplant. This information could be used to assign a risk score to a given epitope load or, in the case of clearly defined immunogenic epitopes, avoid mismatching them entirely. In turn, long-term graft survival may be improved through the minimization of *dn*DSA development.

This study examined a prospective cohort of consecutive renal transplant patients from a single institution, 45 of whom developed *dn*DSA against Class II HLA (DR or DQ). Epitope matching was assessed against traditional matching methods as a predictor for the development of *dn*DSA posttransplant. Models of epitope mismatch load were developed for HLA-DR and HLA-DQ to predict *dn*DSA risk, and specific epitope mismatches were evaluated for their relative immunogenicity.

Methods

Patient population

Approval was obtained from the Institutional Health Research Board (H2011: 211). Three hundred ninety-two consecutive patients received renal transplants at the Health Sciences Centre, Winnipeg, Manitoba between January 1999 and December 2008. One hundred six patients were excluded for pretransplant DSA (n = 30), death with a functioning graft (n = 22), moved and lost to follow-up (n = 14), primary nonfunction (n = 11), and no available sample for high-resolution HLA donor and/or recipient typing (n = 29) leaving 286 patients (adult n = 247, pediatric n = 39) for analysis (Figure 1). This cohort was largely Caucasian (72%) but also included Aboriginal (16%), Asian (9%) or African-American (2%) patients. Standard immunosuppression consisted of a calcineurin inhibitor (tacrolimus, n = 237; cyclosporine, n = 48), mycophenolate mofetil (n = 285) and prednisone (n = 285). Induction therapy with thymoglobulin (n = 29) or basiliximab (n = 56) was used in 85/286 (30%) patients. There was one transplant recipient with an HLA identical twin living donor not treated with immunosuppressive medication.

Adherence

Nonadherence was defined as patient admission of medication nonadherence documented by clinic staff and/or drug levels below the detectable limit. Repeated failure to attend clinic visits or perform laboratory evaluations (i.e. blood draws for medication levels) constituted a pattern of behavior defined as nonadherence in a minority of patients.

Antibody monitoring

The collection and testing of serum samples in this cohort have been detailed previously (2). In brief, serum samples were collected and stored at 0, 1, 2, 3, 6, 12, 18 and 24 months, then yearly, or at the time of biopsy for graft dysfunction, as routine clinical practice in our program since 1990. DSA screening was performed using FlowPRA^{TM} beads representing HLA-A, -B, -C, -DR, -DQ and -DP antigens (One Lambda, Canoga Park, CA). If positive, determination of HLA antibody specificities was performed using FlowPRA^{TM} single antigen Class I and II beads (One Lambda). HLA antibody specificities were confirmed using LABScreen^{TM} single antigen beads using a threshold mean fluorescence intensity (MFI) $\geq$300 (One Lambda).

HLA typing and epitope mismatch identification

High-resolution HLA typing was performed using sequence-specific oligonucleotide probes or sequence-specific primer technology (LABType®

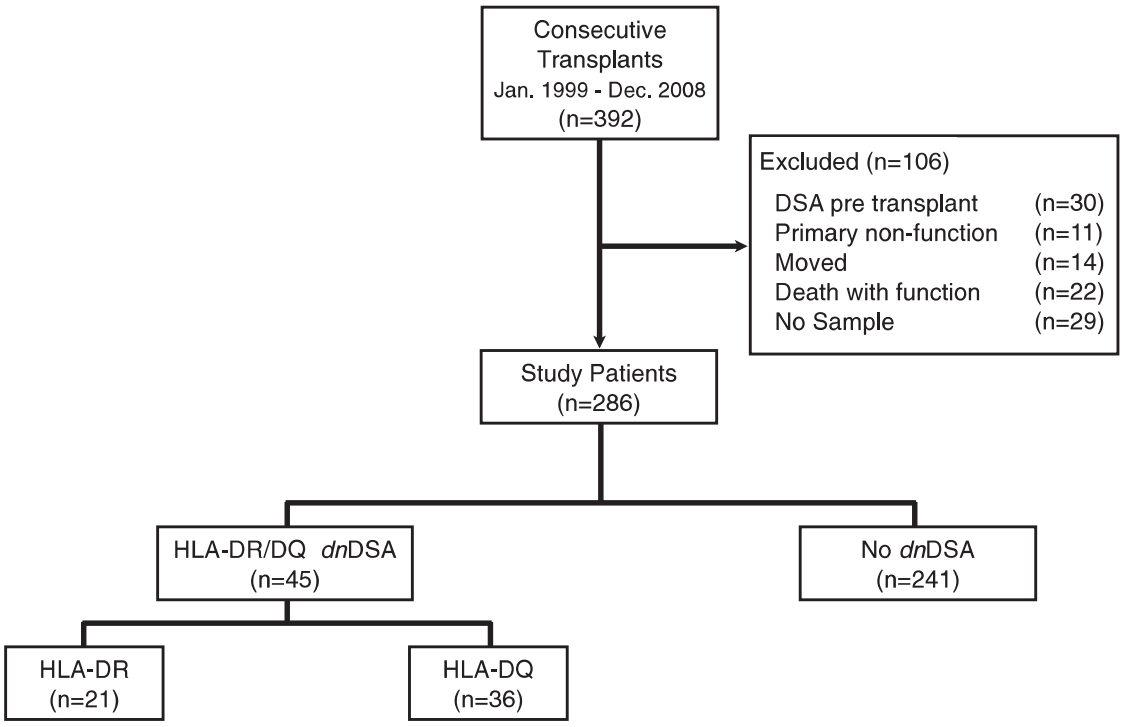

Figure 1: Patient flow.

HD SSO, Micro SSP™; One Lambda). HLAMatchmaker software (HLAMatchmaker DRDQDP Matching version 3.0, http://www.hlamatchmaker.net) was used to define potential epitope mismatches between donors and recipients (13). In brief, this method is based on two underlying principles: (1) the immune system recognizes and develops antibodies against nonself-antigens, or more specifically the epitopes on those antigens, while ignoring self-antigens/epitopes; (2) epitope binding affinity is largely determined by a small number of polymorphic amino acids near the center of the epitope. The evidence and the rationale for these postulates have been reported previously (13). HLAMatchmaker compares amino acid sequences between donor and recipient alleles to identify and quantify differences. Only polymorphic amino acids are of interest and only amino acids at or near the molecule's surface accessible to antibody binding are considered. HLAMatchmaker version 3.0 incorporates the three-dimensional location of these amino acids on the HLA structure and identifies surface exposed patches of polymorphic amino acids that are continuous or discontinuous in the linear sequence but are brought into proximity on the tertiary structure. Patches of polymorphic amino acids on the surface are called eplets. By these methods a catalogue of potential eplet-derived epitopes has been described for each HLA locus (170 HLA-DR$\beta_{1/3/4/5}$, 89 HLA-DQα_1, 76 HLA-DQβ_1, 17 HLA-DPα_1 and 43 HLA-DPβ_1) that may be present on the alleles. We used HLAMatchmaker DRDQDP Matching (version 3.0) software to identify the subset of eplet-derived epitope mismatches that were present in each donor–recipient pair.

De novo *donor-specific epitope specificity assignment*

For transplant recipients who developed *dn*DSA, HLAMatchmaker Single DRDQ allele antibody screen version 2.0 was used to analyze LABScreen™ single antigen bead reactivity patterns to determine specificities of eplet-derived epitopes (http://www.hlamatchmaker.net) (14). In brief, the negative single antigen beads, which by definition have not bound the antibody, and the eplets represented by the alleles found on these beads were excluded as potential candidates for eplet specificity. This also eliminated many eplet candidates from the repertoire represented by alleles on the positive beads. We then examined the positive beads, and the eplet mismatches characterized by the alleles on these beads, for eplets present across all positive beads, which could explain the pattern of bead reactivity. When two or more eplet sites were possible (because they always occur together on the positive beads) they are separated by a slash (e.g. 52PQ/84EV).

A second approach to HLA epitope assignment based on the established knowledge of observed antibody binding reactivity patterns was also used to correlate with the HLAMatchmaker eplets (15,16). Briefly, El-Awar et al (15) isolated alloantibodies or monoclonal antibodies generated by hybridoma cells lines and tested them against LABScreen™ single antigen beads. The amino acid sequences of the alleles on all beads were analyzed to look for surface exposed polymorphic amino acids that were exclusively shared on the beads which reacted with the antibody in question. These amino acids define the putative epitopes and have been called Terasaki epitopes (TerEps). Final assignment of epitope specificity to *dn*DSA in our study integrated HLAMatchmaker eplet-derived epitopes with corresponding TerEps after sequence alignment of reactive beads using the Immunogenetics project HLA database along with published reports of eplet/TerEp correlation (17–21). Three-dimensional models of HLA structures were created and analyzed using freely available Cn3D software (http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml).

Statistical analysis

Stepwise nominal logistic regression was performed to search for significant clinical predictors of *dn*DSA. Nonparametric univariate analysis of patient demographics were compared using the Wilcoxon Rank Sums test. *De novo* DSA free survival analysis was done by the Kaplan–Meier method using the log-rank test for significance. Receiver operating curve analysis was used to determine the optimal eplet-derived epitope mismatch threshold which best predicted *dn*DSA development. Logistic regression was used to assess the significance of individual eplet-derived epitope mismatches as predictors of *dn*DSA.

Results

The patient cohort represented a low risk group overall with 97% receiving their first transplant and 89% of patients having a cPRA <10%. During a median follow-up of 6.9 years, 45 out of 286 (16%) patients developed HLA-DR *dn*DSA (n = 9, Table 1), HLA-DQ *dn*DSA (n = 24) or both (n = 12) at a mean onset of 55 months (median 48, range 6–130 months). The median MFI on initial detection was 5266 (range 776–18 282), and all patients were monitored serially to ensure persistence of the DSA. While three patients had an initial MFI < 1000 (776, 890, 995), two of these individuals had biopsy-proven AMR and the third's subsequent MFI increased to 1224. No patients developed HLA-DP *dn*DSA. Our analysis was performed exclusively in patients who developed Class II *dn*DSA; however, Class I HLA *dn*DSA coexisted with Class II *dn*DSA in 14 out of 45 (31%) patients, which was associated with a nonsignificant trend toward worse graft survival (39% vs. 61%, $p = 0.2$). At the time of initial detection the MFI of Class II *dn*DSA was significantly greater than the MFI of Class I *dn*DSA whether it occurred in isolation or in combination with Class I *dn*DSA (Table S1).

Patients who developed Class II *dn*DSA were younger (31 vs. 42 years, $p < 0.01$, Table 1), had younger donors (35 vs. 40 years, $p < 0.02$) and were more likely to have received a deceased donor graft (62% vs. 47%, $p = 0.05$) compared to those who did not develop Class II *dn*DSA. Nonadherence (n = 39/286) was more common in those who developed Class II *dn*DSA (47% vs. 8%, $p < 0.001$). There was no significant difference with regard to transplant number, cPRA or delayed graft function between the two groups. Median 10-year graft survival was significantly lower in those who developed Class II *dn*DSA (44% vs. 93%, $p < 0.001$).

To quantify HLA-DRβ mismatches HLA-DRβ_1 and HLA-DR$\beta_{3/4/5}$ were considered together for a total high-resolution HLA-DR$\beta_{1/3/4/5}$ mismatch score, which could range from zero to four. Similarly, both HLA-DQα_1 and β_1 chains were considered as a combined HLA-DQα_1/β_1 score, which could range from zero to four mismatches. Both high-resolution and epitope mismatches were greater in the Class II *dn*DSA group compared to the no Class II *dn*DSA group (Table 1). However, on subgroup analysis high-resolution typing revealed a trend, but no significant difference in locus-specific mismatches for those who developed HLA-DR *dn*DSA alone (2.4 vs. 1.8, $p = 0.1$, Table 1) or HLA-DQ *dn*DSA alone (2.3 vs. 1.9, $p = 0.2$) compared to those who did not develop *dn*DSA. In contrast, locus-specific epitope mismatch was significantly greater for those developing

Table 1: Clinical demographics

	No Class II *dn*DSA (n = 241)	Class II *dn*DSA (n = 45)	DR *dn*DSA alone (n = 9)	DQ *dn*DSA alone (n = 24)	Both DR and DQ *dn*DSA (n = 12)
First transplant	98%	96%	100%	95%	92%
Adult recipient	89%	71%*	78%	71%*	67%
Recipient age (years)	42 ± 16	31 ± 17*	32 ± 16	30 ± 15**	34 ± 22
Donor age (years)	40 ± 14	35 ± 15*	38 ± 18	35 ± 15	32 ± 13*
Living donor	53%	38%*	11%*	46%*	42%
Calculated panel reactive antibody (cPRA)	6%	4%	4%	2%	7%
HLA-DR$\beta_{1/3/4/5}$ high-resolution mismatch	1.8 ± 1.2	2.1 ± 0.9*	2.4 ± 0.9	1.8 ± 0.9	2.6 ± 0.7*
HLA-DR$\beta_{1/3/4/5}$ epitope mismatch	13.2 ± 13.5	17.6 ± 10.6**	21.4 ± 8.4*	12.8 ± 9.6	24.2 ± 9.9**
HLA-DQα_1/β_1 high-resolution mismatch	1.9 ± 1.4	2.3 ± 0.8*	2.0 ± 1.0	2.3 ± 0.7	2.7 ± 0.9*
HLA-DQα_1/β_1 epitope mismatch	17.3 ± 16.7	24.4 ± 12.1***	11.0 ± 10.5	27.5 ± 11.7***	28.2 ± 6.0**
HLA-DPα_1/β_1 high-resolution mismatch	1.2 ± 1.1	1.4 ± 0.9	1.7 ± 1.0	1.1 ± 0.9	1.6 ± 0.8
HLA-DPα_1/β_1 epitope mismatch	5.7 ± 6.6	6.9 ± 5.9	9.3 ± 4.1*	5.9 ± 5.7	7.2 ± 4.7
Rejection episodes preceeding *dn*DSA	0.2 ± 0.6	0.6 ± 0.8*	0.8 ± 1.0**	0.4 ± 0.6*	0.8 ± 0.8***
*dn*DSA onset (months)	–	55 ± 34	79 ± 37	51 ± 20	45 ± 33
Delayed graft function	12%	11%	22%	4%	17%
Cold ischemic time (h)	7 ± 6	9 ± 6*	12 ± 6*	7 ± 6	9 ± 6*
Nonadherence	8%	47%***	33%*	46%*	58%***
Median 10-year graft survival	94%	50%***	43%***	64%**	30%***

Plus-minus values, means ± SD.
*dn*DSA, *de novo* donor-specific antibody; HLA, human leukocyte antigen.
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$ compared to no *dn*DSA group.

HLA-DR *dn*DSA alone (21.4 vs. 13.2, $p < 0.02$) or HLA-DQ *dn*DSA alone (27.5 vs. 17.3, $p < 0.001$). For patients who developed *dn*DSA against both HLA-DR and DQ loci, high-resolution mismatches were significantly elevated for both HLA-DR (2.6 vs. 1.8, $p < 0.02$) and HLA-DQ (2.7 vs. 1.9, $p < 0.05$), which was also the case for the epitope mismatch at both HLA-DR (24.2 vs. 13.2, $p < 0.01$) and HLA-DQ (28.2 vs. 17.3, $p < 0.01$) loci. No patients in the study developed *dn*DSA against HLA-DP, and there was a low number of high-resolution (1.2 ± 1.1) and epitope mismatches (5.9 ± 5.7). Low-resolution, high-resolution and epitope mismatch were each analyzed for their ability to predict *dn*DSA development in Figure 2 where epitope mismatch was found to provide better resolution compared to the other methods.

In a multivariate model, independent clinical predictors of HLA-DR *dn*DSA were nonadherence (OR 6.0, $p < 0.001$, Table 2A), HLA-DR epitope mismatch (OR 1.06 per mismatch, $p < 0.001$) and clinical rejection episodes preceding *dn*DSA onset (OR 2.6 per rejection episode, $p < 0.001$). The independent predictors of HLA-DQ *dn*DSA were nonadherence (OR 8.5, $p < 0.001$), HLA-DQ epitope mismatch (OR 1.04 per mismatch, $p < 0.001$) and younger age (OR 1.03 per year younger, $p < 0.01$).

The effect of different epitope mismatch load cutoffs was analyzed using Kaplan–Meier *dn*DSA free survival curves (Figure 3). Dividing epitope mismatch by quartiles revealed a significant difference in *dn*DSA free survival for both HLA-DR ($p < 0.01$, Figure 3A) and HLA-DQ ($p < 0.01$, Figure 3B), which was driven by the difference between the first or second quartile compared to either of the third or forth quartiles. Figures 3(C) and (D) show locus-specific epitope mismatches analyzed using an optimal cutoff, determined by a receiver operating curve analysis, of 10 mismatches for HLA-DR ($p < 0.001$) and 17 mismatches for HLA-DQ ($p < 0.001$). In contrast, apart from a zero antigen mismatch at each locus, attempts to define a threshold using high-resolution typing for HLA-DRβ_1, HLA-DR$\beta_{3/4/5}$, HLA-DQα_1 or HLA-DQβ_1 to minimize *dn*DSA development were uninformative (Figure S1).

Patients were monitored with serial serum tests and the first positive serum for each patient was used to assign epitope specificities for *dn*DSA against HLA-DR or HLA-DQ (Table 3). When analyzing the entire cohort the most common epitope specificities assigned to HLA-DR *dn*DSA were 14SEH (TerEp #1006) assigned to four *dn*DSA, 48YQ (no TerEp defined) assigned to three *dn*DSA and 71DRA/71DEA (TerEp #1018) assigned to three *dn*DSA. A multivariate logistic regression model identified 14SEH (OR 1.7, 95% CI 1.1–2.5, $p < 0.01$) and 71DRA/71DEA (OR 2.6, 95% CI 1.1–5.6, $p < 0.02$) as independent predictors of *dn*DSA while 48YQ (OR 2.4, 95% CI 0.8–6.3, $p = 0.08$) did not reach significance. In a subset analysis of adherent patients (n = 247, Table 2B), 71DRA/71DEA (OR 4.0, 95% CI 1.4–10.4, $p < 0.01$) and 48YQ (OR 4.7, 95% CI 1.2–16.6, $p < 0.02$) were independent predictors of *dn*DSA while 14SEH was no longer significant (OR 1.6, 95% CI 0.9–2.8,

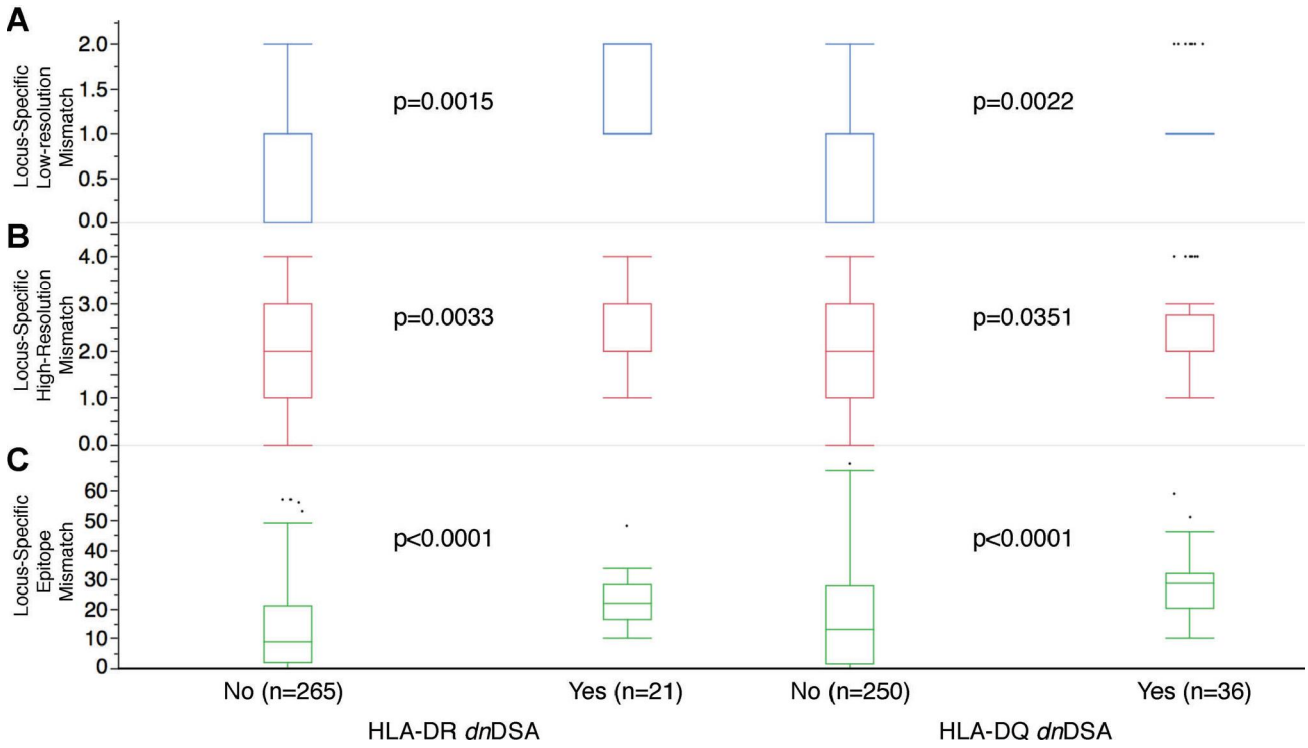

Figure 2: HLA typing methods to predict *dn*DSA development posttransplant. Panel (A) shows the number of low-resolution HLA-DRβ_1 or HLA DQβ_1 mismatches for patients who developed or did not develop *dn*DSA posttransplant. Panel (B) shows the HLA-DR$\beta_{1/3/4/5}$ or HLA-DQα_1/β_1 high-resolution mismatches. Panel (C) shows the HLA-DR$\beta_{1/3/4/5}$ or HLA-DQα_1/β_1 eplet-derived epitope mismatches. *dn*DSA, *de novo* donor-specific antibody; HLA, human leukocyte antigen.

$p = 0.09$). However, it was noted that 4 out of 4 patients who developed HLA-DR *dn*DSA against the 14SEH epitope were nonadherent.

When analyzing the entire cohort the most common epitope specificities assigned to HLA-DQ *dn*DSA were 52PQ/84EV (TerEp #2004) assigned to nine *dn*DSA, 52PL/140T/182N (TerEp #2014) assigned to five *dn*DSA and 45GE/52LL/71RKA (TerEp #2001) assigned to five *dn*DSA. All three of these epitope specificities were significant independent predictors of HLA-DQ *dn*DSA; 52PQ/84EV (OR 2.2, 95% CI 1.2–3.8, $p < 0.01$); 52PL/140T/182N (OR 2.1, 95% CI 1.0–4.3, $p < 0.05$); 45GE/52LL/71RKA (OR 2.5, 95% CI 1.0–5.8, $p < 0.05$). In the subset of adherent patients ($n = 247$) 52PQ/

Table 2: Multivariate models of *dn*DSA predictors[1]

Model	HLA Loci	Predictors	Odds ratio (per unit change)	Odds ratio (over entire range)	p-Value
A: Clinical predictors	HLA-DR	Nonadherence	6.0 (2.1–17.0)	–	<0.001
		HLA-DR epitope mismatch load	1.06 (1.03–1.10)	32.8 (4.6–258.7)	<0.001
		Clinical rejection preceding *dn*DSA	2.6 (1.5–4.6)	120.4 (7.9–2138.0)	<0.001
	HLA-DQ	Nonadherence	8.5 (3.6–20.0)	–	<0.001
		HLA-DQ epitope mismatch load	1.04 (1.0–1.02)	14.0 (2.9–70.7)	<0.001
		Younger age	1.03 (1.0–1.10)	8.9 (1.7–47.9)	<0.01
B: Epitope predictors (adherent patients, n = 247)	HLA-DR	48YQ (TerEp# undefined)	4.7 (1.2–16.6)	22.1(1.5–276.3)	<0.02
		14SEH (TerEp#1006)	1.6 (0.9–2.8)	7.3 (0.5–63.9)	NS
		71DRA/71DEA (TerEp#1018)	4.0 (1.4–10.4)	15.6 (2.0–108.8)	<0.01
	HLA-DQ	45GE/52LL/71RKA (TerEp#2001)	0.7 (0.04–3.4)	0.4 (0.001–11.4)	NS
		52PL/140T/182N (TerEp#2014)	3.0 (1.2–7.4)	9.2 (1.4–52.3)	<0.02
		52PQ/84EV (TerEp#2004)	2.4 (1.1–4.9)	5.8 (1.3–24.1)	<0.02

[1]Odds ratios with 95% confidence intervals. *dn*DSA, *de novo* donor-specific antibody; HLA, human leukocyte antigen.

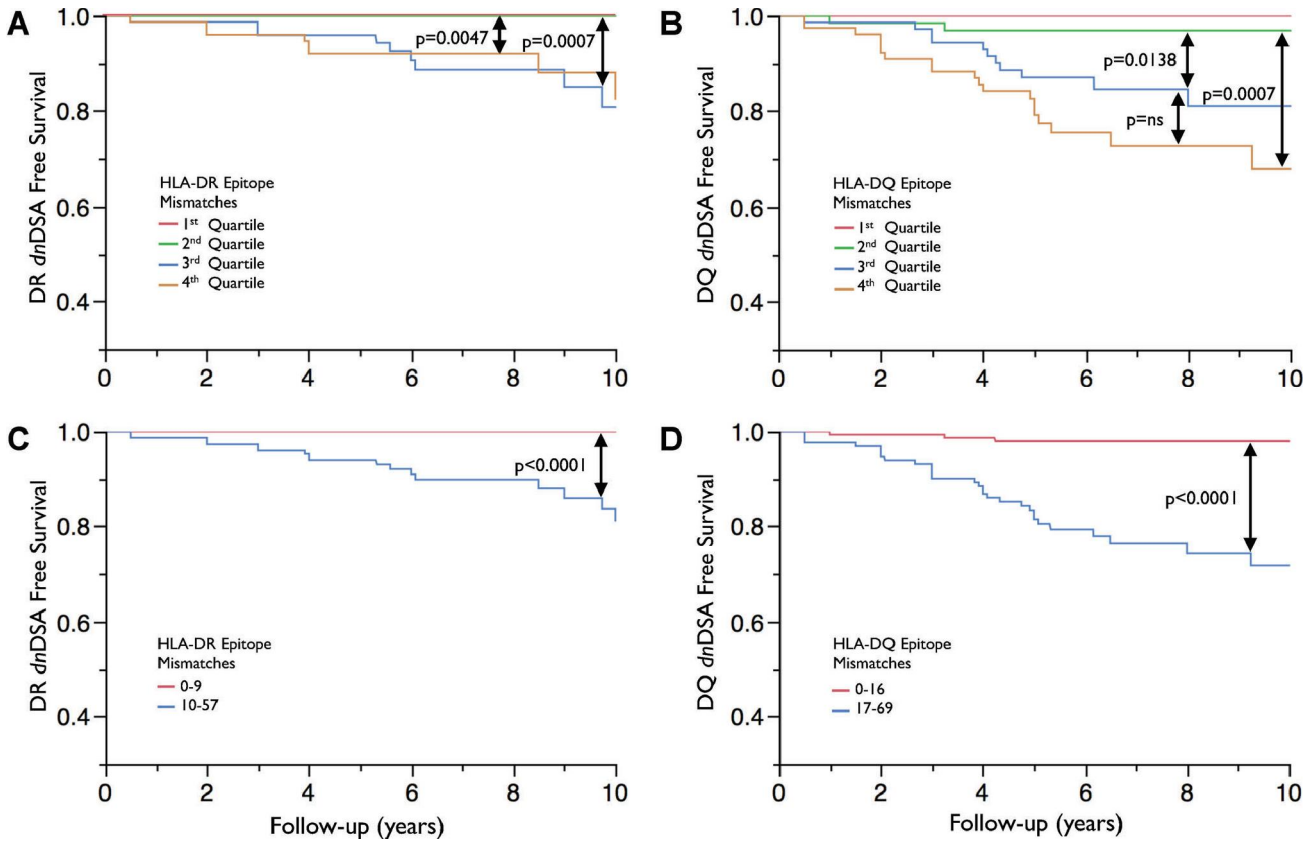

Figure 3: Kaplan–Meier *de novo* DSA free survival curves. Panel (A) shows DR *dn*DSA free survival split by HLA-DR$\beta_{1/3/4/5}$ epitope mismatch quartiles. Panel (B) shows DQ *dn*DSA free survival split by HLA-DQα_1/β_1 epitope mismatch quartiles. Panel (C) shows DR *dn*DSA free survival split by an optimal mismatch cutoff of 10 mismatches for HLA-DR$\beta_{1/3/4/5}$ and in Panel (D) an optimal mismatch cutoff of 17 for HLA-DQα_1/β_1. *dn*DSA, *de novo* donor-specific antibody; HLA, human leukocyte antigen.

84EV (OR 2.4, 95% CI 1.1–4.9, $p < 0.02$, Table 2B), 52PL/140T/182N (OR 3.0, 95% CI 1.2–7.4, $p < 0.02$) were still significant predictors of *dn*DSA while 45GE/52LL/71RKA (OR 0.7, 95% CI, 0.04–3.4, $p = 0.7$) was no longer significant. However, 4 out of 5 patients who developed HLA-DQ *dn*DSA against 45GE/52LL/71RKA were non-adherent. The three-dimensional location of the epitopes found to be significant in the adherent patients is shown on the HLA-DR/DQ surface in Figure 4.

Discussion

The principal findings of this study are that an epitope-based mismatching approach outperforms traditional low-resolution or high-resolution antigen mismatching as a predictor for Class II *dn*DSA development and that some epitopes appear to be more immunogenic than others. While current antigen mismatching is limited by describing two HLA molecules as either matched or mismatched, epitope mismatching affords a more detailed assessment of the degree of difference between the donor and the recipient. Indeed, there was no significant difference in the high-resolution mismatch between those who developed HLA-DR *dn*DSA alone or HLA-DQ *dn*DSA alone. However, the level of epitope mismatch was significantly higher for patients who developed *dn*DSA against either loci, and both HLA-DR and HLA-DQ epitope mismatch were elevated when patients developed antibodies against both loci (Table 1).

Due to the limited range of possible values using traditional whole-molecule mismatch (0, 1 or 2 per locus), identifying patients at low risk for Class II *dn*DSA development required a mismatch threshold of zero at each locus (Figure S1). However, using epitopes to determine an optimal mismatch demonstrated that for the 134 patients with <10 HLA-DR epitope mismatches, none developed HLA-DR *dn*DSA and only 4 out of 145 (2.7%) of patients with <17 HLA-DQ epitope mismatches developed HLA-DQ *dn*DSA after a median follow-up of 6.9 years (Figure 3). Interestingly high-resolution typing affords no additional benefit over low-resolution typing when it comes to predicting the development of *dn*DSA. Thus, spending additional time and money to perform routine high-resolution HLA typing seems to have little benefit unless those results are used as part of an epitope matching strategy.

Table 3: Epitope specificities of *dn*DSA on the initial positive result

*dn*DSA	Eplet-derived epitope	Terasaki epitope
DR13, DR52, DR53	14SEH, 4Q	TerEp #1006, TerEp #1001
DR17	14SEH	TerEp #1006
DR17	14SEH	TerEp #1006
DR13	14SEH	TerEp #1006
DR9	26KHY	TerEp #1401
DR53	48YQ	Undefined
DR53	48YQ	Undefined
DR53	48YQ	Undefined
DR11	57DE	TerEp #1017
DR8, DR12	71DRA/71DEA	TerEp #1018
DR13	71DRA/71DEA	TerEp #1018
DR11	71DRA/71DEA	TerEp #1018
DR15, DR51	71QAA, 108T	TerEp #1020, TerEp #1402
DR15, DR51	71QAA, 108T	TerEp #1020, TerEp #1402
DR17	71QKG	TerEp #1026
DR53	71RAE	TerEp #1023
DR52	98QS	TerEp #1036
DR16	142M	TerEp #1603
DR15	142M	TerEp #1603
DQ7	45EV	TerEp #2005
DQ7	45EV	TerEp #2005
DQ5	45G[1]	TerEp #2009
DQ8	45GV	TerEp #2010
DQ2	45GE/52LL/71RKA	TerEp #2001
DQ2	45GE/52LL/71RKA	TerEp #2001
DQ2	45GE/52LL/71RKA	TerEp #2001
DQ2	45GE/52LL/71RKA	TerEp #2001
DQ2	45GE/52LL/71RKA	TerEp #2001
DQ4, DQ7	46VY[2]/52P[3]	TerEp #2003
DQA1*O3	47QL/52FRR/187T	TerEp #2019
DQ7	52PL/140T/182N	TerEp #2014
DQ7	52PL/140T/182N	TerEp #2014
DQ4	52PL/140T/182N	TerEp #2014
DQ9	52PL/140T/182N	TerEp #2014
DQ4	52PL/140T/182N	TerEp #2014
DQ6	52PQ/84EV	TerEp #2004
DQ5	52PQ/84EV	TerEp #2004
DQ6	52PQ/84EV	TerEp #2004
DQ5	52PQ/84EV	TerEp #2004
DQ5	52PQ/84EV	TerEp #2004
DQ5	52PQ/84EV	TerEp #2004
DQ5	52PQ/84EV	TerEp #2004
DQ6	52PQ/84EV	TerEp #2004
DQ6	52PQ/84EV	TerEp #2004
DQ7	55PPP	TerEp #2006
DQ7	55PPP	TerEp #2006
DQ5	55PR[4]	TerEp #2007
DQ5	70GA/1161	TerEp #2015
DQ5	70GA/1161	TerEp #2015
DQA1*O5	75SL	Undefined
DQ2	77DR	TerEp #2026
DQ2	84QL	TerEp #2013
DQ6	125GQ	Undefined
DQ2	Undefined	Undefined

*dn*DSA, *de novo* donor-specific antibody.
[1]This epitope is characterized by 45G; however, permissible surrounding amino acids include 46E/V.
[2]This epitope is characterized by 46VY; however, permissible surrounding amino acids include 45E/G.
[3]This epitope is characterized by 52P; however, permissible surrounding amino acids include 53L/Q.
[4]This epitope is characterized by 55PR; however, permissible surrounding amino acids include 56L/P.

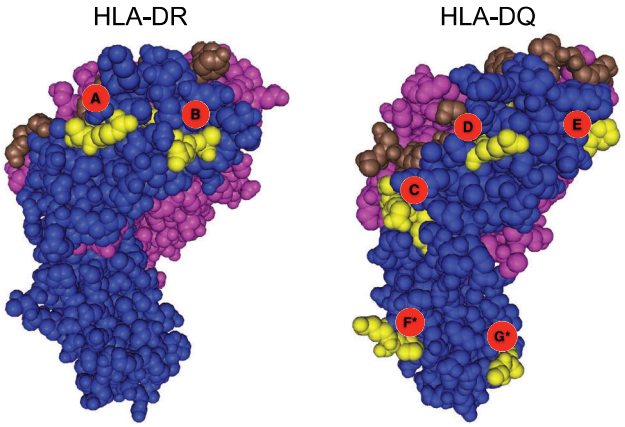

Figure 4: Three-dimensional HLA models with immunogenic epitopes. Amino acids highlighted in yellow are eplet-derived epitopes. These epitope specificities were the most commonly identified epitopes in the LABScreen™ bead analysis based upon bead reactivity patterns and were significant independent predictors of *dn*DSA development in the multivariate model of adherent patients. A (71DRA, TerEp#1018), B (48YQ, TerEp# undefined), C (84EV, TerEp#2004), D (71RKA, TerEp#2001), E (52PL, TerEp#2014; 52PQ, TerEp#2004; 52LL, TerEp#2001), F (182N, TerEp#2014), G (140T, TerEp#2014). *Eplets E, F and G always occur together, therefore, the relevance of eplets F and G in terms of their antibody accessibility given their proximity to the cell membrane is uncertain. Blue, beta chain; pink, alpha chain; brown, peptide. *dn*DSA, *de novo* donor-specific antibody; TerEp, Terasaki epitope.

Another limitation of current algorithms is that matching for HLA-DR does not ensure that the linkage disequilibrium with HLA-DQ is sufficient to prevent HLA-DQ *dn*DSA from arising. Multiple studies have now shown that HLA-DQ *dn*DSA occur more commonly than HLA-DR *dn*DSA (2,3,22–25) and are associated with AMR, transplant glomerulopathy and allograft failure (3,22). In the current study patients who developed isolated HLA-DQ *dn*DSA had significantly elevated HLA-DQ epitope mismatch loads. Therefore, novel strategies whose aim is to prevent the development of Class II *dn*DSA should take into account both HLA-DR and HLA-DQ loci. Indeed, defining a threshold for HLA-DR and HLA-DQ epitope mismatch load could allow one to assign allocation points favoring low mismatch loads for both loci rather than for just a zero HLA-DR or DQ high-resolution mismatch. The epitope mismatch load may also provide clinicians with a more detailed assessment of immunologic risk posttransplant to aid in clinical decision making regarding immunosuppressive sparing strategies or the need for posttransplant monitoring for *dn*DSA. Although the mechanism for how epitope load increases the risk of *dn*DSA development is unknown, the probability of allorecognition by a specific B cell clone likely increases with an increasing number of mismatches, as would the likelihood of an immunodominant epitope being present.

Epitope–paratope interactions are influenced by the size, shape and electrochemical properties of the amino acids

present on the surface of the antigen and antibody (26–28). Thus, it is not surprising that an *in silico* method of eplet-derived epitope assignment based on polymorphic amino acid positions on the three-dimensional surface of HLA models, such as HLAMatchmaker, would have varying degrees of antigenicity or immunogenicity. Indeed, Laux et al (29) defined six HLA-DP epitopes based on hypervariable amino acid regions and found that certain epitopes were more immunogenic than others and that mismatches at these epitopes outperformed low-resolution HLA-DP matching for predicting graft survival in retransplants. Using an early version of HLA Matchmaker, Dankers et al (30) showed that the number of Class I amino acid triplet mismatches correlated with the likelihood of Class I DSA at the time of graft failure. Duquesnoy et al (14) reported the eplet HLA specificities of a heterogeneous group of 75 failed lung, heart, kidney, small bowel, liver and pancreas transplants with Class II DSA. However, the heterogeneity of the population, the lack of a control group that did not develop DSA and the assessment after graft failure make it difficult to assign a reliable risk to any individual eplet-derived epitope mismatch. Kosmoliaptsis et al (31) also reported that both the number of amino acid mismatches and their physiochemical properties were predictive of Class II antibody production. However, this study focused on highly sensitized patients pretransplantation. Our study cohort consisted of a consecutive series of patients without pretransplant DSA, during an era of modern immune suppression that developed *dn*DSA prior to graft failure. In this context, it was possible to identify eplet-derived epitopes to which *dn*DSA were putatively directed (Table 3) and, using the group that did not develop *dn*DSA as a control, some of these same epitopes were significant independent predictors of *dn*DSA development in a multivariate model. Importantly, five out of six of these immunogenic epitopes correlated with known monoclonal antibody or isolated alloantibody single antigen bead reactivity patterns used to define the TerEps (17). If validated in future studies, this information could be used to help clinicians avoid high-risk epitope mismatches at the time of transplant, thus minimizing the risk of *dn*DSA development and improving long-term graft outcomes.

Nonadherence has been shown to be one of the strongest predictors of *dn*DSA (2). Therefore, we examined a subset of adherent patients and found that four out of six of the most commonly assigned epitope specificities were also significant independent predictors of locus-specific *dn*DSA in this group (Table 2B). The fact that these epitopes are associated with *dn*DSA in the presence of immunosuppressive therapy further support their potential immunodominance. Alternatively two of the six commonly assigned epitope specificities were only significant independent predictors in nonadherent patients. Presumably, once patients are off immunosuppression, due to nonadherence or physician withdrawal after graft failure, the immune system is unencumbered from responding to all mismatched epitopes, even those that are relatively weak alloantigens.

Limitations of this study include the relatively small sample size and the associated risk for type II error; thus, risk quantification should be interpreted with caution. However, the fact that Class II epitope mismatch can be demonstrated to be highly significant in this context suggests that the observed signal is real and strategies to minimize epitope load may have a tangible impact. The relatively small sample size may also account for the observation that combined Class I and II *dn*DSA had a nonsignificant trend toward worse graft outcome whereas a larger cohort would have likely detected significance. It is interesting that in these patients the MFI of the Class II *dn*DSA is significantly greater than the Class I *dn*DSA. However, one cannot attribute a dominant role for Class II *dn*DSA in these patients based on this MFI difference alone. Second, though highly suggestive given the retrospective nature of the study, we cannot prove a causal link between improved outcomes and epitope matching. Third, the relatively limited ethnic diversity may restrict the observed immunodominant repertoire of HLA Class II epitopes, highlighting the opportunity to identify novel immunogenic epitopes in other populations.

Conclusion

To minimize the risk for developing *de novo* Class II DSA, the solid organ transplant community should reconsider the construct that a donor mismatched HLA molecule is a single entity. Viewed through the lens of the immune system, it is clear that allorecognition can generate antibodies to multiple nonself-epitope mismatches even on a single molecule. In this context, HLA-DR and -DQ epitope matching appears to outperform traditional low-resolution antigen-based matching and has the potential to reduce the risk of developing *de novo* Class II DSA, thereby improving long-term graft outcome. Alternatively, a strategy that focuses on avoiding a small number of highly immunogenic Class II epitope mismatches may be the optimal approach to minimize risk while maximizing equitable access for all individuals. The latter will require a concerted effort by the transplant community to further inventory and validate which Class II epitopes are immunodominant.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1: Kaplan–Meier *de novo* DSA free survival curves analyzed by locus specific high-resolution mismatch.

Table S1: MFI values by *dn*DSA Class at initial detection.