Acceptable mismatching at the class II epitope level: the Canadian experience

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Purpose of review
To summarize the evidence concerning human leukocyte antigen (HLA) epitope mismatch analysis as a means to predict donor-specific antibody (DSA) development and allograft survival.

Recent findings
HLA epitope mismatch analysis outperforms traditional whole molecule antigen mismatch for predicting the risk of de-novo DSA development. By analyzing the number of epitope mismatches for a given donor-recipient pair, thresholds have been identified to stratify patients into those at high or low risk of de-novo DSA development. Epitope specificity assignment in patients who develop de-novo DSA compared with controls who do not provides an opportunity to study the relative immunogenicity of mismatched HLA epitopes.

Summary
Recognizing that de-novo DSA is a major cause of graft loss, HLA epitope mismatch analysis is a strategy to minimize de-novo DSA development and improve long-term graft survival.

Keywords
antibody-mediated rejection, donor-specific antibody, epitope, kidney transplantation, transplant rejection

INTRODUCTION

Long-term graft survival in renal transplantation continues to be limited primarily by immune-mediated injury and, in particular, chronic antibody-mediated rejection (cAMR) [1,2,3▪]. Donor-specific antibody (DSA) can be avoided at the time of transplant in most patients with highly sensitive assays; however, de-novo DSA develops in 25–30% of low-risk patients within 10 years post-transplant, and this number can be two-fold higher in patients with medication nonadherence [3▪,4]. De-novo DSA has been identified as one of the strongest independent predictors of graft loss which reflects the lack of effective treatment strategies able to prevent or reverse allograft injury secondary to DSA [3▪,5]. Risk factors for de-novo DSA in renal transplantation include human leukocyte antigen (HLA) mismatch, early cellular rejection, and under-immunosuppression [3▪,6▪▪]. However, traditional whole molecule HLA mismatch is constrained by a small range of possible values (0, 1, or 2 per locus) which limit the definition of a precise threshold, other than zero mismatch, under which patients are at low risk of de-novo DSA development.

In the last decade, the combined advances in genetics, single-HLA allele detection beads, and three-dimensional protein modeling have made it possible to examine HLA mismatching at the level of the epitope [7–10]. By dividing the HLA molecule into a compilation of epitopes, the degree of mismatch between two alleles can be defined more precisely, and individual epitopes can be assessed for their propensity to lead to DSA development. This review will focus on recent advances in the field of HLA epitope matching at the class II (HLA-DR, HLA-DQ, and HLA-DP) loci and their potential relevance in transplantation, whereas an accompanying article in this issue will address the HLA class I locus.

THE SIGNIFICANCE OF HUMAN LEUKOCYTE ANTIGEN MISMATCH AT THE CLASS II LOCI

Although de-novo DSA occurs against both class I and class II HLA, it has been noted that majority of

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KEY POINTS

- De-novo DSA development is the strongest predictor of graft failure in low-risk renal transplant patients.
- Predicting de-novo DSA development can be done more precisely using HLA epitope mismatch load, compared with traditional whole molecule antigen mismatch.
- Highly immunogenic epitopes can be identified and could potentially be avoided at the time of organ allocation.

de-novo DSA are class II [3▪,11–17,18▪,19–21], tend to occur late post-transplant [3▪,11,13,20,22,23], and respond poorly to therapy [24]. In a low-risk cohort of 315 renal transplant recipients with more than 6 years follow-up, we found that 72% of patients with de-novo DSA developed either HLA class II or a combination of HLA class I and II de-novo DSA. Furthermore, studies that have attempted to define risk factors for de-novo DSA development have consistently identified class II, but not class I HLA, antigen mismatches as univariate or multivariate predictors [3▪,4,12]. This is consistent with the historical observation that HLA-DR mismatch has a stronger effect on graft survival compared with HLA class I alleles [25–27], and the more recent observation that the most common de-novo DSA are those developed against the HLA-DQ locus [3▪,13,19,20,28,29].

Because the alpha and beta chain of HLA-DQ both contain amino acid polymorphisms (unlike HLA-DR in which the alpha chain is polymorphic), it is not surprising that multiple reports have found that a higher number of epitope mismatches exist between HLA-DQ alleles compared with those found between HLA-DR alleles [6▪▪,30]. In our own study, the median number of epitope mismatches was significantly higher at the HLA-DQ locus compared with the HLA-DR locus (15 vs. 10, $P < 0.002$). Although one might assume that matching for HLA-DR also results in adequate matching at the HLA-DQ locus because of their linkage disequilibrium, the high prevalence of HLA-DQ de-novo DSA in an era when most programs prioritize for HLA-DR matching refutes this. In two recent studies, antibody-mediated rejection, transplant glomerulopathy, and allograft failure have been correlated with HLA-DQ de-novo DSA which strongly supports the pathogenicity of these antibodies [19,28]. Therefore, minimizing class II HLA-DR and DQ mismatches may reduce class II de-novo DSA development and, in turn, increase long-term allograft survival.

De-novo DSA against HLA-DP appears to occur less frequently. In our study of 315 patients, no patients developed HLA-DP de-novo DSA after a mean follow-up of more than 6 years [6▪▪]. This can be easily understood by the observation that the median number of epitope mismatches at the HLA-DP locus in our study was four (range 0–28), which was less than half the number at the HLA-DR locus and only one-third as many as the HLA-DQ locus. Thus, the limited epitope diversity at the HLA-DP locus may account for the infrequent development of HLA-DP de-novo DSA.

CLASS EPITOPE MISMATCH PREDICTS HUMAN LEUKOCYTE ANTIGEN ANTIBODY DEVELOPMENT

Using HLAMatchmaker software (HLAMatchmaker DRDQDP Matching version 3.0, http://www.hlamatchmaker.net) Duquesnoy *et al.* [30] were the first to study the correlation between HLA class II epitope mismatch and the likelihood of sensitization in 75 failed kidney, liver, heart, lung, pancreas, and small bowel transplants. DSA was more common in those with a higher number of epitope mismatches at the HLA-DRβ_1 loci (8.5 vs. 6.2 mismatches, $P = 0.003$), as well as DR$\beta_{1/3/4/5}$ combined (21.4 vs. 10.6 mismatches, $P < 0.0001$). However, the correlation between epitope load and sensitization at the HLA-DQ locus was not reported. In a study of 30 intestinal transplant recipients, Gerlach *et al.* [31] evaluated the sum of both HLA class I and II epitope mismatches and found that de-novo HLA antibody development was more likely in those with a greater number of mismatches (64 vs. 45, $P = 0.01$).

Using a slightly different approach, Kosmoliaptsis *et al.* [32] studied the number of polymorphic amino acid mismatches in a group of 30 sensitized wait-listed patients known to have HLA antibodies, against 17 different HLA-DR and seven different HLA-DQ serologic specificities. As the number of HLA-DR or DQ polymorphic amino acid mismatches increased, the frequency of alloantibodies against HLA-DR or DQ also increased [odds ratio (OR) 3.85 per mismatch, $P < 0.001$]. Furthermore, using the mean fluorescence intensities (MFI) from the LABScreen single-antigen bead assay (One Lambda, Canoga Park, CA), a median regression analysis revealed a significant relationship between the number of polymorphic amino acid mismatches and the median MFI values for the specificities ($P < 0.0001$). These findings highlight that although as little as one amino acid mismatch can be immunogenic and lead to DSA development, an increased number of nonself amino acids may lead to a more robust immune response.

EPITOPE MISMATCH PREDICTS DE-NOVO DONOR-SPECIFIC ANTIBODY DEVELOPMENT

Given the strong correlation between class II de-novo DSA and graft failure [3▪,5], we designed a study to evaluate the benefit of using epitope mismatch to predict de-novo DSA development [6▪▪]. The cohort studied consisted of 286 low-risk donor-recipient pairs without DSA at the time of transplant in which 45 developed class II de-novo DSA (HLA-DR $n=9$, HLA-DQ $n=24$, or both $n=12$) with a median follow-up of 6.9 years. High-resolution typing was performed on all donors and recipients, and HLAMatchmaker was used to determine epitope mismatches at the HLA-DR$\beta_{1/3/4/5}$, HLA-DQ$\alpha_1\beta_1$, and HLA-DP$\alpha_1\beta_1$loci. The main findings were that epitope mismatch outperformed whole molecule antigen-based mismatch as a predictor of de-novo DSA development and that certain epitopes were more immunogenic than others. HLA locus-specific epitope mismatch was elevated in those who developed de-novo DSA at both the HLA-DR (21.4 vs. 13.2 mismatches, $P<0.001$) and HLA-DQ (27.5 vs. 17.3 mismatches, $P<0.001$) loci compared with those that did not develop de-novo DSA. However, locus-specific high-resolution HLA mismatch was not predictive of de-novo DSA development at the HLA-DR (2.4 vs. 1.8, $P=0.1$), or HLA-DQ (2.3 vs. 1.9, $P=0.2$) loci. Furthermore, when patients developed both HLA-DR and HLA-DQ de-novo DSA, both loci had significantly increased epitope mismatch [HLA-DR (24.2 vs. 13.2 mismatches, $P<0.01$), HLA-DQ (28.2 vs. 17.3 mismatches, $P<0.01$)]. Epitope mismatch was also a significant independent predictor of de-novo DSA in a locus-specific multivariate model of de-novo DSA development for both HLA-DR (OR 1.06 per mismatch, $P<0.001$) and HLA DQ (OR 1.04 per mismatch, $P<0.001$) after adjustment for medication adherence and previous rejection episodes.

A receiver-operating characteristic (ROC) curve analysis was utilized to define optimal mismatch thresholds to minimize risk for HLA-DR and HLA-DQ de-novo DSA development. Using an HLA-DR epitope mismatch threshold of 10, we found that recipients whose epitope mismatch was above the threshold developed significantly more HLA-DR de-novo DSA (14% vs. 0%, $P<0.001$). Similarly, above the HLA-DQ epitope mismatch threshold of 17, patients developed significantly more HLA-DQ de-novo DSA (24% vs. 3%, $P<0.001$). Because of the limited range of values that can be mismatched using traditional whole molecule HLA antigen mismatch (0, 1, or 2 per locus), we found that only zero whole molecule antigen mismatches could be considered low risk. Patients with a traditional whole molecule HLA antigen mismatch of one were already significantly more likely to develop de-novo DSA compared with those with zero mismatches at both HLA-DR (9% vs. 0%, $P<0.01$) and HLA-DQ (19% vs. 0%, $P<0.01$) loci. These findings highlight the difficulty in identifying a safe mismatch threshold other than zero using traditional methods.

A second component of the study was to evaluate the immunogenicity of individual epitopes using the 241 patients who did not develop de-novo DSA as the control group [6▪▪]. The potential epitope mismatches for each HLA class II locus were quantified using HLAMatchmaker-defined epitopes for all donor-recipient pairs and then used as predictors of de-novo DSA development in a multivariate model [33]. In the entire cohort, we identified two HLA-DR epitopes 14SEH (OR 1.7, $P<0.01$); 71DRA/71DEA (OR 2.6, $P<0.02$) and three HLA-DQ epitopes 52PQ/84EV (OR 2.2, $P<0.01$); 52PL/140T/182N (OR 2.1, $P<0.05$); 45GE/52LL/71RKA (OR 2.5, $P<0.05$) that were independent predictors of locus-specific de-novo DSA development. However, after restricting the analysis exclusively to adherent patients ($n=247$), we found that some epitopes fell out of the model, whereas others became independent predictors. Thus, we identified two HLA-DR epitopes 71DRA/71DEA (OR 4.0, $P<0.01$); 48YQ (OR 4.7, $P<0.02$) and two HLA-DQ epitopes 52PQ/84EV (OR 2.4, $P<0.02$); 52PL/140T/182N (OR 3.0, $P<0.02$) that were independent predictors of locus-specific de-novo DSA development in recipient's adherent to their immunosuppression. The observation that immunogenicity of epitopes can vary in the setting of under-immunosuppression may explain the inconsistency observed between epitopes described as immunogenic in previous cohorts of failed transplants or wait-listed patients compared with those identified during the course of a functioning transplant [6▪▪,30,34].

When considering epitope immunogenicity under-representation of certain epitopes may be possible because of limited ethnic diversity within the cohort. Our cohort was predominantly whites (72%), but included Aboriginal (16%), Asian (9%), and African-American (2%) recipients. Thus, the opportunity exists to identify other potentially immunogenic epitopes in novel cohorts. High-resolution HLA typing was performed on all donors-recipient pairs in our study above; however, previous studies have relied on predictive algorithms to covert serologic (low-resolution) HLA typing results into the high-resolution HLA typing required for epitope assignment. Although these algorithms have been shown to have a high level of accuracy, it is likely that the small differences are missed for patients with rare alleles and that some populations are

under-represented. For example, in a Chilean study by Silva *et al.* [35], only 62 out of 173 eligible patients could be studied because of a high frequency of alleles not recognized by the converting program in this particular ethnic cohort. This highlights the need for molecular high-resolution typing in cohorts with ethnicities under-represented in current HLA databases in order to conduct reliable epitope analysis.

CLASS II HUMAN LEUKOCYTE ANTIGEN EPITOPE MISMATCH PREDICTS ALLOGRAFT SURVIVAL

Although epitope mismatch at the HLA class I loci has been correlated with allograft survival in a number of reports [35–39], only a few studies to date have evaluated the utility of epitope matching at the class II Laux *et al.* [40] analyzed the effect of HLA-DP epitope matching on 1478 failed renal transplant patients who had been retransplanted. By dividing the polymorphic portion of 19 HLA-DPβ_1 alleles into six regions, each containing three to seven epitope variants, they reported that repeat transplant patients with more than three epitope mismatches had reduced 2-year graft survival compared with those with less than two epitope mismatches (65 vs. 77%, $P < 0.0001$). Interestingly, patients with a traditional HLA-DPβ_1 antigen mismatch of two, but with less than three epitope mismatches, had better survival than patients with only one HLA-DPβ_1 traditional antigen mismatch with more than two epitope mismatches (77 vs. 66%, $P < 0.049$). Thus, epitope mismatch at the HLA-DP locus outperformed traditional whole-molecule antigen-based mismatch for predicting allograft survival in this cohort of repeat renal transplants.

Haririan *et al.* [37] were the first to use HLAMatchmaker to investigate the effect of class II epitope mismatch on survival in a cohort of 101 predominantly African-American (94%) renal transplant recipients. In a subset of 76 patients in whom HLA-DR and DQ typing was available, triplet mismatch was found to be a significant predictor of allograft survival for the HLA-DQ (OR 1.9 per mismatch, $P = 0.029$), total HLA-DR/DQ triplet mismatch (OR 1.13 per mismatch, $P = 0.045$), and total class I and II combined triplet mismatch (OR 1.11 per mismatch, $P = 0.034$) at 40-month post-transplant. In our cohort, the combined HLA class II epitope mismatch (HLA-DR/DQ/DP) was significantly elevated in patients with graft loss (46.8 vs. 36.1, $P = 0.025$). Locus-specific HLA mismatch was also increased in those with graft failure at the HLA-DR (17.3 vs. 13.0, $P = 0.047$) and HLA-DP (8.3 vs. 5.6, $P = 0.004$) loci, with a trend toward greater mismatches at the HLA-DQ (21.2 vs. 17.5, $P = 0.192$) locus.

CONCLUSION

Given the established link between antibody-mediated allograft injury and premature graft loss in transplantation, and the lack of adequate therapies with proven efficacy, novel methods for minimizing DSA development are critical [5]. Epitope mismatch load at the HLA class II loci in renal transplantation can predict the development of sensitization post-transplant, de-novo DSA development during the course of a functioning transplant, and allograft survival. In addition, an organ allocation strategy that focuses on avoiding a small number of highly immunogenic class II epitope mismatches may help minimize risk of de-novo DSA development. Post-transplant knowledge of the precise epitope mismatch load may be valuable to guide and individualize clinical care with regards to immunosuppression protocols, antibody surveillance strategies, or the need for protocol biopsies.

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Conflicts of interest

There are no conflicts of interest.

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