Molecular Mismatch and the Risk for T Cell–Mediated Rejection

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T cell–mediated rejection (TCMR) is evidence of alloimmune reactivity characterized by inflammation within the tubules, interstitial space, and/or arterial intima and media in kidney transplantation. Risk factors for TCMR include younger recipient age, delayed graft function, inadequate immunosuppression, and HLA mismatch.[1-6] Advances in genetics and 3-dimensional modeling have led to a renaissance in human leukocyte antigen (HLA) mismatch assessment through the evaluation of such mismatches at the molecular level. Since HLA molecules can differ from as little as 1 to more than 50 amino acids, even within the same locus, quantifying differences at the molecular level has the potential to improve precision in HLA mismatch assessment. In the literature, the most commonly used techniques to quantify HLA molecular mismatch are the HLA Matchmaker eplet method[7] or amino acid mismatch quantification and characterization.[8,9] Eplets are small clusters of surface-exposed amino acids that include at least 1 non-self residue. At about 3 Å in diameter, eplets are the approximate size of the binding site of an antibody's complementary-determining region. Consequently, eplets are essentially the smallest functional unit of an antibody paratope-epitope interface. Several publications to date have shown a correlation between TCMR and HLA-DR/DQ molecular mismatch. In 2019, we showed that HLA-DR/DQ molecular mismatch (using the HLA Matchmaker approach) could be used to generate alloimmune risk categories that independently correlated with Banff grade 1A or worse TCMR, de novo donor-specific antibody (DSA) development, and antibody-mediated rejection (ABMR).[10] In a follow-up publication in 2020, we expanded this analysis to show that HLA-DR/DQ molecular mismatch also correlated with Banff borderline TCMR.[11] Recently, while examining the relationship between persistent or subsequent TCMR and allograft survival, we found that HLA-DR/DQ molecular mismatch correlated with both number of TCMR episodes and the severity of Banff TCMR grade.[5] In addition, Bestard et al[12] studied a cohort of low-immunological-risk kidney transplant recipients and found that in recipients with negative results on a pretransplant interferon-γ ELISPOT assay, HLA class II molecular mismatch (determined by HLA Matchmaker) significantly correlated with TCMR.

Related Article, p. 718

In this issue of *AJKD*, Senev et al[13] report on the association between HLA molecular mismatch and TCMR using an alternative evaluation of HLA molecular mismatch as provided by the PIRCHE-II algorithm. This approach is based on a widely used computational algorithm called NetMHCIIpan, which enables the prediction of peptide binding affinity by HLA class II based on a neural network method trained on a large set of experimentally derived data.[14] A recent update of NetMHCIIpan (version 4.0) enables the ranking of a query peptide affinity compared to natural peptide binders for a specific HLA molecule, in an attempt to remove bias emanating from the variable peptide-binding preferences of different HLA.[15] PIRCHE-II implements a previous iteration of NetMHCIIpan (version 3.0) that enables the enumeration of putative CD4-positive T-cell epitopes derived from polymorphic parts of a donor HLA.[16] The PIRCHE-II score is the sum of all donor-derived candidate peptides with a predicted binding affinity to recipient HLA class II of less than 1,000 nM. It is important to note that PIRCHE-II employs a relatively weak binding affinity threshold (strong binders often have half maximal inhibitory concentration [IC_{50}] values <100 nM); this combined with the algorithm's avoidance of peptide binding affinity ranking when identifying immunogenic peptides means that a high number of polymorphic donor HLA peptides are accepted as potential T-cell epitopes and consequently, the PIRCHE-II score correlates with amino acid sequence–based algorithms.[17]

The study by Senev et al examined a retrospective single-center cohort of 893 kidney transplant recipients with surveillance biopsies at 3 and 12 months. In a multivariate analysis, they found that donor HLA-DRB1 and HLA-DQB1 PIRCHE-II scores had the strongest association with TCMR and with allograft survival, while class I (HLA-A, B, C) HLA molecular mismatch was not associated with these end points. The correlation with TCMR was consistent across both early and late TCMR episodes. Biopsies showing mixed rejection were included as acute TCMR, whereas biopsies with ABMR alone were not considered a histological end point. Sensitivity analysis excluding recipients with DSA (pretransplant or de novo) or C4d-positive ABMR showed similar findings to the full cohort and, interestingly, suggested an additional association between donor HLA-DP PIRCHE-II score and acute TCMR. Strengths of the study include the large sample size and the use of the latest version of the PIRCHE-II software (the first to account for donor peptide presentation by recipient HLA-DRB3/4/5, DQA1B1, and DPA1B1 molecules in the analysis). In addition, the subset analysis after exclusion of pre- and post-transplant DSA provides confidence that the correlation observed between HLA molecular mismatch and TCMR exists independently of DSA.

Drawbacks of the Senev et al cohort include the limited patient population diversity (98.3% White Europeans) and

that only 85% of recipients studied were on modern tacrolimus, mycophenolate-based therapy. Furthermore, the TCMR analysis was censored at the time of the last biopsy; however, the timing of the biopsies was not provided. Most importantly, it should be noted that the mechanistic basis of the association between donor HLA molecular mismatch scores and TCMR reported by this study is predicated on the assumption that the PIRCHE-II algorithm specifically assesses the indirect allorecognition pathway and therefore priming of donor-specific CD4-positive T cells. Nevertheless, the authors have previously reported in the same patient cohort similar associations between HLA-DR and DQ antigen mismatching and risk of TCMR, and between HLA-DQ eplet load and TCMR (HLA-DRB1 eplet load was not specifically assessed).[18] Similarly, in the current study, HLA-DRB1/3/4/5 and DQB1 eplet load was associated with early acute TCMR. The association between donor HLA-DQ eplet load and death-censored graft survival has also been previously reported by the authors. Unfortunately, a formal comparison of models including eplet versus PIRCHE-II molecular mismatch scores is not provided and it is not clear whether the PIRCHE-II score associations specifically reflect an assessment of CD4-positive T-cell alloreactivity or just the degree of donor HLA dissimilarity (polymorphism) compared to recipient HLA. The low stringency for peptide binding prediction used in PIRCHE-II (as acknowledged by the authors) and the known correlation between eplet and PIRCHE-II scores suggest that the latter might be the case.[17] Finally, given the previously reported association of HLA class II antigen mismatching with TCMR in this cohort, it would have been valuable if this study had assessed whether HLA molecular mismatching provides a superior assessment of TCMR risk compared to classical HLA mismatching.

Although there has been debate regarding whether TCMR contributes to graft loss independent of ABMR in the modern era of potent immunosuppression, this and other recent publications have pushed TCMR, including Banff borderline TCMR, back into the spotlight. Thus, understanding the key risk factors that predispose recipients to experience TCMR events is a long overdue pursuit. We, and others, have shown that in patients treated with modern tacrolimus and mycophenolate-based therapy, a first TCMR event is independently associated with increased risk for de novo DSA development and death-censored and all-cause graft loss.[5] Furthermore, a second TCMR event, adjusting for baseline covariates and time-dependent covariates (delayed graft function, first TCMR, and ABMR), is associated with an even greater increased hazard for both death-censored and all-cause allograft loss. Using tools such as HLA class II molecular mismatch will be critical to enriching future trials of novel/augmented immunosuppression protocols with recipients at increased immunological alloimmune risk to avoid over-immunosuppression in those at low risk for alloimmune responses.

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