Novel aspects of epitope matching and practical application in kidney transplantation

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This review describes the recent developments in the applicability and clinical significance of epitope matching in kidney transplantation. As incremental human leukocyte antigen (HLA) mismatches are associated with increased risk of rejection and allograft loss, HLA-matching remains one of the standard immunological triage tests to determine transplant suitability. Advancements in tissue-typing techniques have led to innovative concepts of HLA-matching at the epitope level. Epitopes are configurations of polymorphic amino acid residues that are recognized by B cells, and antibodies reactive with these epitopes lead to rejection and/or premature allograft loss. Even though there is substantial advancement in the characterization and understanding of epitopes, evidence supporting the added clinical benefit of epitope matching over traditional antigen matching and the ability to identify clinically relevant immunogenic epitopes remains poorly defined. We present an overview of the evidence surrounding the immunogenicity of mismatched donor epitopes and the applicability of HLA epitope matching in kidney transplantation. A better understanding of the immunogenicity and structural characteristics of HLA epitopes will guide clinicians to integrate epitope matching as an important parameter for donor selection in kidney transplantation.

Kidney International (2018) **93,** 314–324; http://dx.doi.org/10.1016/j.kint.2017.08.008

KEYWORDS: acute rejection; chronic kidney disease; epitope; histocompatibility; kidney transplantation; tissue typin

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Received 26 May 2017; revised 30 July 2017; accepted 3 August 2017; published online 20 October 2017

The human leukocyte antigen (HLA) system plays an essential role in the regulation of the body's immune system to counteract pathogens through antigen presentation and the recognition of "self" and "nonself." It makes use of a group of cell-surface antigen-presenting proteins classified as class I and II major histocompatibility complex molecules. Humans have 3 class I (A, B, C) antigens that are present on all nucleated cells and 8 class II (DPA1/B1, DQA1/B1, DRB1, DRB3/4/5) antigens that are present only on antigen-presenting cells and endothelial cells. The heterodimers HLA-A, -B, and -DRB1 contribute to the majority of the polymorphisms and therefore have been the predominant focus of HLA matching in the allocation of donor kidneys for transplantation. Matching at the HLA-ABDR loci remains the cornerstone of deceased donor kidney allocation because of the association between increasing numbers of HLA-ABDR mismatches and incremental risk of rejection and graft loss after kidney transplantation.[1] In addition, HLA mismatches predispose to the development of donor-specific anti-HLA antibodies (DSAs), which are strongly linked to antibody-mediated rejection (AMR) and late allograft loss.[2,3] The presence of DSAs is often an impediment to repeat transplantation, with highly sensitized patients experiencing a longer waiting period for a suitable donor kidney compared with unsensitized patients. HLA-DQ matching is not routinely considered in the allocation of donor kidneys, but it is assumed that close HLA-DR matching has reduced the likelihood of HLA-DQ mismatches. However, a recent registry study of 788 kidney transplant recipients showed that in those who received zero HLA-DR mismatched kidneys, 11% of recipients will still have any mismatches at the HLA-DQ locus. In this study, recipients who received HLA-DQ mismatched kidneys have a 50% greater risk of the development of acute rejection, including AMR, independent of mismatches at the HLA-ABDR loci.[4]

The evolution of our understanding of the HLA system is closely linked to advancements in technology. Traditional serology-based HLA typing methods can be completed relatively quickly but are dependent on the availability of specific cell types, viability, and appropriate antisera. The emergence of molecular HLA typing techniques over the past 2 decades has allowed for a more specific, flexible, and robust means of high-resolution HLA typing. Data

generated via the genome project and the initiation of polymerase chain reaction techniques further refined DNA-based techniques for HLA-typing, which has led to the development of a number of polymerase chain reaction–based techniques currently in use.

Role of epitope matching in kidney transplantation

Every HLA molecule is composed of a unique set of serologic epitopes made of polymorphic amino acid residues, and it is these structures and their conformation and position that determine antibody accessibility, recognition, and subsequent reactivity. Different HLA molecules can share individual epitopes and therefore epitopes that are not present on self-HLA molecules are considered foreign by the immune system. HLAMatchmaker (http://www.epitopes.net) is a computer algorithm that determines HLA compatibility between donors and recipients by assessing the 3-dimensional molecular modeling of the epitope-paratope interfaces of antigen-antibody complexes (Figure 1).[5] The earlier version of the program considered each HLA as a string of continuous short linear sequences of polymorphic amino acid residues on the molecular surface called triplets,[5] but the updated eplet version considers longer and often discontinuous sequences of polymorphic amino acid in antibody-accessible positions (Figure 2).[5,6] The "theoretical" eplets that have already been defined are listed in the web-based HLA epitope registry (http://epregistry.ufpi.br/terms/index). By evaluating the position and number of foreign (i.e., nonself) and shared eplets (i.e., self, hence antibodies cannot be induced to self) between donors and recipients, HLAMatchmaker is able to calculate the number of eplet mismatches for each donor-recipient pair. Even though high-resolution 4-digit HLA typing is required, 4-digit alleles can be estimated from 2-digit alleles using the catalog of common and well-documented (CWD) HLA alleles, taking into account the ethnic groups, the most likely allelic typing based on haplotype frequency and the frequencies of the paired haplotypes in the population. The common and well-documented alleles are predominantly derived from the Caucasian population, and therefore patients or donors who are not Caucasian may potentially have an incorrect allele assigned.[7] Entry into the HLAMatchmaker of the extended HLA typing at the HLA-C, -DRB3/4/5, -DP, and -DQA1B1 alleles is essential to analyze differences in amino acid residues between the potential immunizer (i.e., donor) and the HLA alleles of the potential antibody producer (i.e., recipient). Four-digit HLA typing methods are time-consuming, and therefore at present are not practicable in deceased donor kidney allocation in which a rapid turnaround in HLA typing is essential; consequently, 2-digit HLA typing methods remain the standard typing technique for deceased donors. However, high-resolution 4-digit molecular HLA typing methods should be undertaken for all potential kidney transplant candidates, particularly highly sensitized patients in whom the correct assignment of permissible DSAs is critical to determine transplant potential.

Along with advances in the typing of HLA alleles, it is recognized that anti-HLA antibodies may bind to distinct epitopes expressed on each serologically defined antigen. The techniques used to detect these anti-HLA antibodies have evolved from complement-dependent cytotoxicity assays to more sensitive techniques including flow cytometry and solid-phase assays (e.g., enzyme-linked immunosorbent or Luminex assay [Luminex Corporation, Austin, TX), which allows a more accurate assessment of a transplant candidate's immunologic risk pretransplantation.

Clinical significance of anti-HLA DSAs

AMR is recognized as one of the dominant causes of late allograft loss after kidney transplantation,[8] commonly preceded by the presence of DSAs, combined with complement activation leading to subsequent vascular and/or endothelial cell injury. Several epidemiologic studies have shown that the presence of pretransplantation DSAs is associated with up to a 40 times increased risk of acute AMR, transplant glomerulopathy, and late allograft loss, and, therefore, transplant eligibility in patients with preexisting DSAs should be carefully considered.[2] In a probabilistic Markov model, screening with a Luminex assay (using a DSA mean fluorescence intensity [MFI] threshold of 500 to determine suitability) resulted in a savings of more than US$1.1 million, prevented 11 acute rejection episodes within 1 year, 6 episodes of allograft loss over 5 years, and gained 54 quality-adjusted life-years over 20 years for every 100 kidney transplants compared with screening with complement-dependent cytotoxicity alone.[9]

There is increasing evidence that the development of *de novo* DSA posttransplantation, especially DSAs directed against HLA-DQ, is associated with AMR and/or allograft loss.[2,3,10] Detection of DSAs that are capable of binding complement (i.e., C1q-binding DSAs) may further improve determination of kidney transplant recipients at a greater risk of AMR and allograft loss, but it remains unclear whether early intervention or more frequent monitoring of these recipients before the development of antibody-mediated injury is of clinical benefit.[11]

Clinicians should be aware of the limitations when interpreting the results of solid-phase assays. The generated output of a Luminex assay is a numerical value expressed as MFI, which does not provide a quantitative measure of the amount of anti-HLA antibodies in the sera. These MFI results must be interpreted in the context of positive and negative control sera as well as the threshold associated with a positive (flow cytometric) cross-match and, therefore, comparisons of the MFI between laboratories must be undertaken with great caution.[12] Varying antigen density between beads, differences in the preparation of sera for testing, the possibility of false-negative/-positive results, shared epitopes between single-antigen beads, and the potential of multiplicity of single-antigen beads for the different antigens are some of the factors contributing to the difficulty in establishing a clinically relevant MFI