Anti-HLA Antibody: The Role of Epitopes in Organ Transplantation

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Abstract

Recent developments have been achieved for better matching in solid-organ transplantation by epitope matching. HLA matching remains a standard immunologic strategy to determine organ compatibility for recipients. Advancements in tissue typing have introduced HLA matching at the epitope level. For this, B cells are able to recognize polymorphic amino acid configurations, which are named epitopes. However, antibody production against these epitopes may be a leading cause of rejection. Although data to support the added clinical benefit of epitope matching over traditional antigen matching are still lacking, there are at least 2 theoretical reasons for epitope matching: (1) to avoid future allosensitization with the development of anti-HLA antibodies and (2) to allow selection of a suitable allograft for highly sensitized patients through virtual crossmatch. Sensitive antibody tests with a comprehensive panel of single alleles have yielded informative, donor-specific antibody reactivity patterns that can be analyzed with a computer algorithm. This program (HLAMatchmaker) uses structurally defined eplets to describe epitopes that can react with specific antibodies. Although low mean fluorescence intensity levels by Luminex (Austin, TX, USA) assay are usually considered low risk for solid-organ transplant, it could be important in post-transplant humoral rejection. Thus, specific shared eplets should always be investigated with respect to previous transplant mismatches. Epitopes are present on a single HLA (private epitope) or shared by multiple antigens (public epitope). The phenomenon of cross-reactivity in HLA testing, often explained as cross-reactive groups of antigens with antibody, can be clearly explained now by public epitopes, an antibody targeting an epitope that shows positive reaction with all antigens sharing the epitope. A better understanding of the immunogenicity and structural characteristics of HLA epitopes will guide us to consider epitope matching as an important parameter for donor selection in kidney transplant in the near future.

Key words: *Allosensitization, Antibody-mediated rejection, Donor-specific antibody, Eplet*

Introduction

The human leukocyte antigen (HLA) system regulates the human immune system against invasion of pathogens. It is composed of 2 major histocompatibility complex (MHC) molecules: MHC class I and II.

Because of the close relationship between higher risk of rejection and graft loss and an increasing numbers of HLA mismatches, HLA-A, HLA-B, and HLA-DR matching remains the core of deceased organ allocation.[1] In addition, HLA mismatches can predispose to the development of anti-HLA donor-specific antibodies (DSAs), which are strongly linked to antibody-mediated rejection and late allograft loss. The presence of pretransplant DSAs is a great barrier for candidates of second or third solid-organ transplant procedures. These patients remain longer on wait lists until a suitable donor can be found.

Although concern about the degree of HLA matching when ABO blood type and T-cell crossmatch are compatible has been previously low,[2] optimizing the HLA matching for class I and II has been shown now to improve allograft survival.[3] Among class II HLA matching, HLA-DR may be more important than HLA-DQ, whereas the mismatch in the latter may cause acute rejection in up to 50% of cases.[4]

HLA typing is determined by serology or polymerase chain reaction methods. Although the

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serology method can be easily performed, the polymerase chain reaction method allows HLA typing with higher resolution and more precision through a DNA-based technique. High-resolution molecular HLA typing has resulted in increased knowledge of amino acid sequences of HLA alleles. It has enabled us to identify polymorphic positions of the HLA, as well as provided more knowledge about the quaternary structure of the HLA by modeling of crystalline HLA molecular structures.[5] HLA typing has several advantages in solid-organ transplant. It allows improved donor-recipient HLA matching, therefore reducing patient exposure to immunologic alloantigens. With HLA typing, preformed antibodies could also be specified; hence, donors with these HLAs would not be accepted for these highly sensitized recipients or otherwise the plan of desensitization protocols would be implied.

The resolution of molecular typing methods means the ability to distinguish among minor differences in allele sequences, which vary according to the technology used and the number of alleles at a particular locus. Low-resolution or 2-digit typing can distinguish among allele groups and is similar to serologic typing, whereas high-resolution or 4-digit typing can distinguish among alleles to the protein level. The HLA nomenclature Website currently lists 15 000 HLA alleles, encoding more than 10 000 unique proteins.[6]

In addition to HLA typing, testing for anti-HLA antibodies is mandatory to identify presensitized patients. Currently, solid-phase assays are widely available (see details in Sypek and associates[7]). These assays include indirect enzyme-linked immunosorbent assays or microbead flow cytometry assays.[7]

Many centers use population frequencies of HLA typing to determine the number of calculated panel-reactive antibodies based on the specificity of a potential recipient's anti-HLA antibody profile. This number represents the percentage of the donor pool with whom the recipient is predicted to have a positive crossmatch and is increasingly being used in organ allocation algorithms.

Epitope and epitope matching

An epitope refers to the specific target amino acids that an individual antibody binds to. Generally, an epitope is about 5 or 6 amino acids in length. A protein sequence may actually have different epitopes that bind to the paratope. Binding between the paratope and the epitope occurs at the tip of the variable region on the antibody.

Development of a custom antibody is important to allow targeting of a specific epitope on a protein versus simply having antibodies (which may be against a number of epitopes) that recognize a particular protein. Modeling of crystalized HLA molecules and amino acid sequence comparisons between HLA alleles has allowed researchers to view structural descriptions, referred to in the HLAMatchmaker program as eplets, which are essential components of HLA epitopes. Several reviews have described molecular models of HLA epitope structures and their reactivity with antibodies,[8] which was originally termed as a triplet.[9] Eplets are small configurations of amino acids in antibodies that are in accessible locations on the HLA molecular surface. They are recognized by their amino acid sequence numbers and polymorphic residues with standard single letter codes.

Antibody reactivity to a presensitized HLA follows first the recognition of a recipient antibody to the specific donor epitope that is then recognized as the foreign antigen by the recipient immune system. HLA compatibility between recipients and donors can be assessed by 3-dimensional molecular modeling, for epitope-paratope interfaces of antigen-antibody complexes (see details in Lim and associates[10]). This is the fundamental feature of HLAMatchmaker software, which is based on a computer algorithm.[10]

Although high-resolution 4-digit HLA typing usually is mandatory for eplet matching (see example in Sypek and colleagues[7]), 4-digit alleles can be estimated from 2-digit alleles using a catalog of common antigens found within the HLAMatchmaker Website. Four-digit HLA typing methods are time-consuming; therefore, these methods are not presently practical 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 for highly sensitized patients in whom the correct assignment of permissible DSAs is critical to determine transplant potential.[10] With the use of the freely available HLAMatchmaker software, the number of eplet mismatches between a donor and

recipient or the eplet specificity of an anti-HLA antibody can be determined based on 4-digit HLA typing.

Clinical significance of anti-HLA donor-specific antibodies

Anti-HLA DSAs are strongly associated with increased risk of rejection and allograft loss.[1] Although no consensus for defining their pathogenicity and no standard for their evaluation to guide clinical decision making exist, anti-HLA DSAs are considered as important biomarkers for predicting allograft injury and loss. Transplant centers presently evaluate immunologic risk before and after transplant using mean fluorescence intensity (MFI) provided by single-antigen flow bead technique, which demonstrates sensitivity and intensity of anti-HLA antibodies.

Advances have been made in diagnosis of patients with antibody-mediated rejection and the role of anti-HLA antibody in transplant outcomes. In addition to the presence of pretransplant DSAs associated with increased risk of acute antibody-mediated rejection, these may also be associated with transplant glomerulopathy and late allograft loss. Therefore, transplant eligibility in patients with preexisting DSAs should be carefully considered.

Assessment of the capability of anti-HLA antibodies to bind complement, particularly C1q binding, and the characterization of their immunoglobulin G (IgG) subclass composition are important. The tendency of anti-HLA DSAs for binding complement is known to be associated with increased risk of antibody-mediated injury and poor allograft survival in solid-organ transplant. In addition, the type of IgG subclass of anti-HLA DSAs may have a direct relationship with allograft injury and allograft survival. Stratifying patients by their immunologic risk may solve the puzzle of alloimmune conditions determining allograft outcomes and increase long-term allograft and patient survival rates by improving the efficacy of allocation policies and therapeutic strategies.

Systematic monitoring and precise characterization of anti-HLA DSAs, including their complement binding capacity and IgG subclass composition, could add to their predictive value for allograft loss on the basis of their assessment of strength by MFI level.[11] The risk of antibody-mediated rejection and allograft loss could be significantly reduced by avoiding HLA-incompatible transplant across preformed C1q binding and/or IgG3-positive anti-HLA DSAs.

Although the Luminex solid-phase assay (Luminex, Austin, TX, USA) is the method of choice to identify anti-HLA antibodies in recipients, it does not provide a quantitative measure of the amount of anti-HLA antibodies in sera. Reports of MFI results may be different among laboratories, as the standards among laboratories are not similar. Varying antigen density between beads, differences in the preparation of sera for testing, the possibility of false-negative or 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 threshold associated with adverse allograft outcomes.[12] All epitope or eplet mismatches are not able to provoke anti-HLA antibody uniformly. Therefore, we should differentiate immunogenic versus nonimmunogenic eplets based on their antibody stimulation property. With this regard, in vitro verification of those eplets that can bind to antibody is essential to identify potentially immunogenic eplets. The clinical significance of immunogenic eplets is not only dependent on their ability to induce an antibody response (ie, immunogenicity), but the antibodies (including complementary determining regions) must have the capacity to interact and bind to the immunizing eplet and the eplet paired with certain amino acid configurations to form antigen-antibody complexes (ie, antigenicity) that may be capable of inducing complement activation.[10]

Immunogenic eplets can be identified by 2 mechanisms. First, it is possible to use monoclonal antibodies directed at defined HLA molecules. Second, Luminex can be used for antibody absorption and elution methods for recombinant single HLA antigen-expressing cell lines that recognize Terasaki epitopes.

The ability of HLA-specific memory B cells of recipients to induce alloantibodies against HLA class I or II DSAs may improve the risk stratification in predicting the production of adverse kidney allograft outcomes. Antigen-specific B cells can differentiate into memory B and plasma cells through stimulation of T cells. Sensitive methods such as enzyme-linked immunospot assays could be used to detect these HLA-specific memory B cells and thus could stratify

the probability of DSA development (see details in Lim and associates[10]). The frequency of immunoglobulin-secreting B cells can be determined with the enzyme-linked immunospot assay. B cells secreting antigen-specific immunoglobulin, or indeed immunoglobulin of all specificities, can be quantified using this assay.

B-cell antigenic epitopes could be predicted by their physicochemical characteristics. In this method, epitope antigenicities can be speculated by evaluation of the hydrophobicity and propensity for binding of the epitopes to the antibodies. This characteristic defines the antigenicity power of the epitope-paratope interactions. Differences in the number of polymorphic amino acid mismatches and their physiochemical properties for a given recipient HLA type are strong predictors of class II alloantigen immunogenicity and alloantibody response before organ transplant.[13] Among the physiochemical properties, hydrophobic and electrostatic interactions between epitopes and antibodies are more important than the traditional consideration of the number of epitope mismatches.

Although most HLA class II epitopes remain unverified because of their complexities, many HLA class I epitopes have been verified using monoclonal antibodies and adsorption/elution.

Epitope or eplet mismatches and solid allograft outcomes

HLA locus-specific triplet or eplet mismatches are associated with early graft failure by development of de novo DSAs, acute rejection, and/or transplant glomerulopathy. Although the threshold of eplet mismatches for developing adverse allograft outcome has not been established, the goal of HLA matching in transplantation is to reduce the number of foreign targets (including mismatch epitopes) that antibodies may be raised against and to avoid the targets of specific preformed antibodies.

A transplant program in Europe has aimed to prioritize appropriate kidneys for highly sensitized patients.[15] These patients remain on wait lists for extended periods unless a zero class I triplet mismatch donor is found. In patients with a negative flow crossmatch test, even the existence of mismatched HLA-A/B antigens would not be a barrier for transplant.[14] HLAMatchmaker has been used in this program to identify organs, regardless of mismatches at the antigen level, with few eplet mismatches, which are therefore of lower immunologic risk. This Web-based program could significantly expand the potential donor pool for highly sensitized recipients.

Clinical studies have examined the value of eplet mismatches for kidney,[15-20] lung,[21] and heart[22] transplant. All of these studies found a strong positive correlation between the number of class I (or II) triplet mismatches and the proportion of individuals producing anti-HLA antibodies, poor graft function, and glomerulopathy. The studies have also suggested a strategy to minimize eplet mismatches in solid-organ transplant. This strategy could result in a reduction in the rate of anti-HLA antibodies, which would in turn reduce antibody-mediated rejection, graft failure, and sensitization of recipients.

HLA-DR and HLA-DQ epitope matching has the potential to minimize the risk of de novo class II DSA development, thereby improving long-term graft outcomes.[16] Epitope specificity analyses have revealed that 3 HLA-DR and 3 HLA-DQ epitopes were independent multivariate predictors of class II de novo DSAs. HLA-DR and HLA-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 outcomes.

The epitope may also play a role in development of transplant glomerulopathy.[17] Minimization of HLA-DR and HLA-DQ eplet mismatches may decrease the incidence of transplant glomerulopathy diagnosed by indication biopsies. The odds ratios for transplant glomerulopathy were 2.84 and 4.62 in the presence of 27 to 43 and > 43 HLA-DR plus HLA-DQ eplet mismatches versus < 27 eplet mismatches, respectively. When the eplet load was modeled as a continuous variable, the odds ratio for transplant glomerulopathy was 1.25 for every 10 additional HLA-DR plus HLA-DQ eplet mismatches.

All mismatched HLAs should not be considered as harmful for the allograft. Acceptable HLA mismatches are mismatched HLAs at the broad antigen level, comprising structurally and functionally compatible eplets, which are unlikely to be of clinical importance. HLAMatchmaker has identified B-cell epitopes without antibody induction, which are possibly acceptable mismatches. Unacceptable antigens, which are identified by the presence of specific antibodies by complement-dependent cytotoxicity assays in the setting of previous immunizing events such as rejection, will further assist in the selection of an immunologically compatible donor kidney.

Conclusions

There is little doubt that epitope matching will become one of the standard triage tests for future kidney transplant allocation and assessment of immunologic risk. HLA epitope matching will be beneficial for prevention of sensitization, selection of donors for highly sensitized patients, and improvement of transplant outcomes. In addition, understanding the complex interactions between the induced antibody and the reactive HLA molecules will contribute to the identification of acceptable mismatches and virtual crossmatching even for highly sensitized patients. Large-scale collaborative studies should be conducted to confirm the potential utility of HLA epitope-based matching before it can be recommended in widespread clinical practice.

There are several gaps in the literature that must be addressed before potential widespread use of epitope or eplet matching in kidney transplant. First, an association between immunogenic eplet mismatches and clinical outcome must be established, which could assist in the decision of whether to consider immunogenic eplet mismatches rather than the total number of eplet mismatches in allocation and immunologic risk assessment. Second, the benefits and costs of incorporating an alternative eplet-based allocating algorithm should be determined, which would allow an understandings of its effect on transplant potential, benefit/cost, and projected survival of the entire transplant cohort. Third, the benefits and costs of incorporating HLA-DQ matching into the allocating algorithm should be determined, given that HLA-DQ DSA is the predominant de novo DSA occurring posttransplant. Finally, high-resolution HLA typing should be completed for non-white ethnic groups with incorporation of the alleles into the HLAMatchmaker program. These ethnic groups should include indigenous patients and other minorities in whom there is likely to be a high prevalence of novel HLA alleles, which is essential in the accurate identification and calculation of immunogenic mismatched eplets.

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