Towards HLA epitope matching in clinical transplantation

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CHAPTER

2

DEFINING THE IMMUNOGENICITY AND ANTIGENICITY OF HLA EPITOPES IS CRUCIAL FOR OPTIMAL EPITOPE MATCHING IN CLINICAL RENAL TRANSPLANTATION

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ABSTRACT

Transplantation of an human leukocyte antigen (HLA) mismatched graft can lead to the development of donor-specific antibodies (DSA), which can result in antibody mediated rejection and graft loss as well as complicate repeat transplantation. These DSA are induced by foreign epitopes present on the mismatched HLA antigens of the donor. However, not all epitopes appear to be equally effective in their ability to induce DSA. Understanding the characteristics of HLA epitopes is crucial for optimal epitope matching in clinical transplantation. In this review, the latest insights on HLA epitopes are described with a special focus on the definition of immunogenicity and antigenicity of HLA epitopes. Furthermore, the use of this knowledge to prevent HLA antibody formation and to select the optimal donor for sensitized transplant candidates will be discussed.

INTRODUCTION

Human leukocyte antigen (HLA) matching in renal transplantation is an important factor influencing long-term graft survival.[1,2] The chance of finding an unrelated fully matched kidney donor is slim due to the high level of polymorphism of HLA antigens.[3] So far, matching in renal transplantation has mainly been done for HLA-A, -B, and -DR antigens and even then, most recipients receive a graft mismatched for at least one or more HLA antigens. Recipients of a mismatched graft have an increased chance to develop donor-specific antibodies (DSA), which are associated with poor allograft survival.[4-6] Furthermore, the development of DSA complicates repeat transplantation, a problem that is most obvious for highly sensitised patients, who generally experience long waiting times.

Immunisation by a single HLA antigen mismatch can result in antibodies directed against numerous other HLA antigens, which is called cross-reactivity.[7-9] It has previously been shown that this cross-reactivity is caused by sharing of determinants to which an antibody can bind, called epitopes, by various HLA antigens.[9-11] In the early 1990's, HLA antigens that shared an epitope were assigned to cross-reactive antigen groups (CREG)[12,13] and matching based on these CREGs appeared to be associated with a better graft survival.[14] The antibody-reactivity patterns observed in sensitised patients also indicated that a relatively small number of epitopes are involved in antibody induction and that the emerging antibodies are directed against common epitopes.[15,16]

Identifying antibody -or B cell- epitopes on HLA antigens and understanding their immunogenicity and antigenicity will be imperative for the development of novel matching strategies that aim at reduced antibody induction after transplantation, as well as identification of acceptable mismatches for highly sensitized patients. The application of high resolution molecular HLA typing has resulted in an increased knowledge of the amino acid sequences of HLA alleles, enabling the identification of polymorphic positions, as well as a better understanding of the quaternary structure of the HLA by modelling of the crystalline HLA molecule structures.[17-20] These tools have become very useful for defining polymorphic areas harbouring theoretical antibody epitopes. In addition, highly sensitive single antigen bead (SAB) based HLA antibody identification assays have been introduced, providing antibody-reactivity patterns with extensive specificity patterns on the allele level.[21] The latter is of great importance to determine the actual epitopes recognised by HLA antibodies. In this review, we will discuss the latest insights into antibody epitopes and the difference between immunogenicity and antigenicity. A proper definition of the immunogenic HLA

epitopes and understanding the interaction between HLA antigen and antibody is crucial for the improvement of matching strategies in clinical transplantation.

Box 1. Various definitions of epitopes
Eplet/functional epitope: polymorphic amino acid configuration that triggers an antibody response (defined by R. Duquesnoy)
Immunogenic epitope: polymorphism that triggers an antibody response
Structural epitope: all polymorphisms that are covered by an antibody footprint

STRATEGIES TO DEFINE HLA EPITOPES

Theoretical epitopes

Epitopes are defined as parts of an HLA molecule that are recognised by the immune system as foreign, which implies the involvement of amino acids (or amino acid sequences) not present on self-HLA antigens (Box 1). In case of antibody epitopes, the polymorphic amino acids must be at sites that are accessible by an antibody molecule. Antibodies recognise conformational epitopes, which can be both linear strands of amino acids (linear epitope), or amino acids in close proximity in the three-dimensional structure of a molecule (discontinuous epitope). In a pioneering attempt to define antibody epitopes on HLA molecules, Duquesnoy *et al.* used linear amino acid sequences of serologically defined HLA antigens together with known molecular three-dimensional structures to determine polymorphic amino acids at antibody accessible positions for each HLA class I locus.[22] These residues were proposed as being the critical components of an immunogenic linear epitope and resulted in the identification of linear sequences of maximal three polymorphic amino acids at the molecular surface that were referred to as triplets.[22,23]

As mentioned above, antibodies can also recognise epitopes formed by polymorphic amino acids from different parts of the molecule that come into close proximity due to the folding of the protein. Indeed, analyses of the available quaternary HLA molecule structures with Cn3D software (https://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml)[19] clearly show that the triplet concept does not fully cover all theoretical epitopes, as discontinuous polymorphic amino acids form patches, with a radius of approximately 3 angstrom (Å), on or near the molecular surface. Consequently, a cluster of polymorphic amino acids, either linear or discontinuous, within a 3-3.5 Å radius of an antibody-accessible sequence position was defined as being capable of inducing an antibody response and was named an eplet.[24,25] So

far, many theoretical HLA class I and HLA class II eplets have been defined using this method and are listed in the HLA epitope registry (http://epregistry.ufpi.br/terms/index).[26,27]

It has become clear that each HLA allele consists of a unique combination of epitopes, but that the three classical HLA class I loci share certain epitopes. In addition, the location of the polymorphic amino acids of the three classical HLA class I loci turned out to be similar. Consequently, for epitope analysis, HLA class I alleles should not only be compared with alleles from the same locus, called intralocus, but also interlocus by which the alleles are additionally compared with the alleles of the other HLA class I loci. This principle has been applied for almost every HLA class I epitope study. In contrast, the epitopes of HLA class II loci are analysed intralocus due to the fact that HLA-DR has a highly polymorphic beta chain and an oligomorphic alpha chain, while HLA-DQ and HLA-DP molecules have two polymorphic chains. Therefore, the defined clusters of antibody accessible polymorphic amino acids are different between the three loci and thus HLA class II epitope should be analysed separately per locus. Regardless, further studies are necessary to prove that the use of interlocus comparison of HLA class I and intralocus comparison of HLA class II is the optimal way to define the number of eplet/epitope mismatches.

A computer algorithm named HLAMatchmaker was developed by Rene Duquesnoy, originally based on triplets, and later modified for eplets as the critical component of immunogenic epitopes that can elicit an antibody response (http://www.epitopes.net/). An important component of the algorithm is that antibodies cannot be induced against eplets present on a self-HLA antigen.[22,23,25,28] This algorithm can be used to determine for each mismatched donor HLA antigen which eplet is non-self, compared to the repertoire of self-eplets on the HLA antigens of the recipient, resulting in a quantification of eplet mismatches. Additionally, the algorithm can identify eplets involved in antibody-reactivity patterns detected in patient's serum.

Antibody-verified epitopes

Eplets have been defined based on sequence comparisons and available quaternary three-dimensional structures of HLA molecules. However, this does not mean that every eplet defined is indeed able to induce antibodies. These potential antibody-inducing patches need verification by laboratory tests showing antibody binding to these structures. Studies by various groups using monoclonal antibodies (mAbs) directed at defined HLA molecules showed that these reagents are very useful to identify shared antibody eplets between HLA antigens.[29,30] Especially human mAbs have been used in SAB assays to successfully verify

an extensive number of eplets.[31,32] Despite this, many eplets should for the moment be considered as theoretical eplets until it is proven that they are indeed being recognised by alloantibodies.

El-Awar *et al.* used an alternative approach to define antibody-verified epitopes.[33] Alloantibodies and mAbs were absorbed and eluted from recombinant single HLA antigen expressing cell lines and HLA antibody specificity was determined with SAB assays. The amino acid sequences of the reactive HLA alleles were compared and the exposed polymorphic amino acids within an antibody-binding region of approximately 700-900 Å, that were exclusively shared between the reactive HLA alleles, were considered to define the antibody epitope. The number of unique amino acids of these epitopes is between one and four, located in discontinuous positions. The epitopes defined by this method have been named Terasaki epitopes (TerEp).[34-38] As the TerEps are defined by one or a combination of amino acids it can occur that a residue at a specific position of an epitope on the immunising allele is also present on an allele of the recipient, yet this is not defined as a self-epitope as the combination of amino acids of the epitope is different.[39]

Similar to the mAb-verified eplets, for TerEps it has formally been proven that these are targets for antibodies. Likewise, for this method the limited number of mAbs and allosera studied so far makes it likely that the current list of TerEps is not complete. Comparative studies observed a huge overlap between eplets and TerEps, with only a small number of TerEps lacking a corresponding eplet.[40,41] Another limitation of the use of mAbs for the verification of antibody-verified epitopes is the restriction to common HLA alleles. As a consequence, the epitopes that are currently verified are mainly present on common HLA alleles, whereas epitopes on rare HLA alleles remain unverified. This poses a disadvantage for ethnic minorities as it will be more difficult to identify antibody-verified epitopes for these populations.

Other means of verifying epitopes on HLA antigens have been described, but one should be cautious to draw any definitive conclusions from these approaches. In a recent study, postpartum sera of women were used to screen for novel antibody-verified eplets.[42-44] Sera from women after one or two pregnancies were screened with SAB assays and the antibody-reactivity pattern was analysed for new antibody-verified epitopes. In contrast to the TerEp studies, no absorption and elution assays were performed. On basis of this type of reagents it is impossible to rule out that the antibodies are produced by more than one B cell clone, leaving the possibility that multiple epitopes are involved in the final antibody reactivity. Furthermore, analysis of sera obtained after a second pregnancy may be complicated by the

interference of antibodies induced after the first pregnancy by a different haplotype. This emphasises the importance of strict guidelines for verifying epitopes based on antibody-reactivity pattern observed in sera. In our opinion, the only way to formally verify an antibody epitope is by (human) mAbs and/or absorption-elution studies.

IMMUNOGENICITY OF AN ANTIBODY EPITOPE

For a proper use of epitope matching in clinical transplantation, the immunogenicity and antigenicity of epitopes should be defined first. Although these are different characteristics of an epitope, these terms are used often interchangeably, which is not correct. Immunogenicity is the ability to induce an antibody response while antigenicity is based on the actual interaction between an antibody and an antigen, which involves both the polymorphic amino acids comprising the immunogenic epitope and other crucial polymorphic amino acid configurations that act as contact sites.

Induction of antibodies

In theory, any amino acid on a particular position of a donor HLA molecule that is not present on the recipient's HLA molecules has the ability to trigger an antibody response. However, whether an epitope truly induces an antibody response is dependent on the total make up of HLA molecules of the recipient. Dankers *et al.* showed that HLA-A28 positive women who delivered an HLA-A2 positive child more often form antibodies against HLA-A2, while women who have HLA-A2 themselves and delivered an HLA-A28 child did not develop antibodies against this HLA antigen. These observations could be explained by an immunogenic epitope that is unique for HLA-A2. Thus, when HLA-A2 is a self-antigen, the unique immunogenic epitope is self and will not trigger an antibody, while in case of an HLA-A2 mismatch the epitope is more likely to trigger an antibody response.[45]

In population studies, various groups have shown (a) correlation between the number of epitope mismatches between donor and recipient, and DSA development, thus demonstrating a quantitative effect of mismatches on the immunogenicity of an HLA molecule. However, one or a few epitope mismatches can be sufficient to induce an antibody response.[46-48] This indicates that immunogenicity is not merely a quantitative issue. The characteristics of the amino acid substitution, in relation to the patient's own HLA molecules, are important for the induction of an antibody. A high number of epitope mismatches can be an indicator for the risk of forming an antibody because the chance is higher that one of these mismatched epitopes

is immunogenic. However, for an individual patient the nature of the epitope mismatch determines if an antibody response is actually triggered.

A prerequisite for an epitope to be immunogenic is that the induced DSA reacts with that particular epitope. Upon transplantation, patients receive maintenance immunosuppression, which may interfere with DSA formation.[49,50] It is conceivable that not every epitope mismatch results in an antibody response due to the immunosuppressive medication. DSA detected in transplant recipient might be restricted to highly immunogenic epitope mismatches that escape the suppressive properties of the medication.[48]

As discussed above, both eplets and TerEps are polymorphic amino acid configurations on donor HLA alleles that are non-self. However, while amino acids on a specific position can be shared between donor and recipient, the combination of amino acids in a configuration can be mismatched. The question is whether such configurations are indeed necessary for antibody induction or if a single amino acid difference between recipient and donor on an antibody accessible position can already determine its immunogenicity. Possibly both scenarios are valid, since some of the eplets and TerEps consist of only a single non-self amino acid.[26,27,37] Recently, Kosmoliaptsis and colleagues compared the number of eplet mismatches and the number of HLA class I and II amino acid mismatches after intralocus and interlocus comparison between donor and recipient alleles as predictive parameters for DSA induction. This population study showed that both the number of eplet mismatches and the number of polymorphic amino acid mismatches are predictors of DSA formation. In this study, no advantage of the eplet approach over the number of amino acid mismatches was observed.[51-53]

Furthermore, the same group demonstrated that physiochemical properties of the amino acid substitution can predict immunogenicity. Each amino acid has unique characteristics, which are determined by the hydrophobicity and electrostatic charge of the side chains. In addition to the number and position of individual mismatched amino acids, the hydrophobicity and electrostatic charge can be determined for each mismatched amino acid, resulting in a hydrophobicity mismatch score (HMS) and electrostatic mismatch score (EMS). Both HMS and EMS appeared to be strong predictors of HLA class I and II *de novo* DSA formation after kidney transplantation.[51,53] The HMS and EMS had superior predictive value for DSA formation compared to eplet mismatches and amino acid mismatches, which warrants further studies.[52]

Nonself-self paradigm

The above-mentioned strategies define immunogenic epitopes as mismatched or foreign epitopes, which are absent on the HLA alleles of the recipient and trigger an antibody

response. Although HLAMatchmaker is also based on this principle, Duquesnoy more recently proposed a nonself-self paradigm to explain some antibody reactivity that otherwise remains unexplained. According to this paradigm, some antibody responses towards foreign HLA cannot solely be induced by an immunogenic eplet mismatch, but require a self-amino acid configuration to be present on the mismatched allele.[54,55] This theory is based on the assumption that B cells with low-affinity immunoglobulin receptors for self-HLA epitopes are present but their affinity for mere self-epitopes is too low to trigger B-cell activation and antibody production. These B cells do become activated when confronted with the combination of a few amino acids which are non-self, and a self-amino acid configuration. The hypothesis implies that the presence of a self-eplet is required to induce an antibody response for a certain group of epitopes. However, not every eplet present in the epitope registry meet the non-self-self criterion and many non-self-eplets are antibody verified.[26,27] While the non-self-self paradigm is interesting, it is difficult to conceive why HLA antibodies would have self-reactive properties. Unlike T cell receptors, B cell receptors are not selected on basis of a low affinity for self HLA antigens. So far, laboratory data confirming the presence of low affinity self-HLA reactive immunoglobulin receptors are lacking. An alternative explanation for the involvement of a self-amino acid configuration on the mismatched donor allele is that HLA molecules share many amino acids. If an amino acid or eplet induces an antibody response, a number of these adjacent nonpolymorphic amino acids will be shared with the patient's own HLA molecules.

T cell epitope

The immunogenicity of antibody epitopes becomes even more complex when considering the requirement for $CD4^+$ T cell help for a full-blown B cell response resulting in class-switched antibodies. Helper T cells play an essential role in the differentiation of B cells into IgG producing plasma cells.[56] After recognition by the B-cell receptor, the target antigen can be internalised and degraded into peptides, finally resulting in peptides being presented in context of self HLA class II on the B cell. The presence of peptides that can be presented by the particular self-HLA class II molecules determines whether B cell clones receive T cell help. Upon cognate interaction between B and T cell, enhanced CD40 ligand expression and cytokine production by the T cell results in B cell differentiation into plasma cells. Indeed, it has been shown that the HLA class II phenotype of the recipient determines the immunogenicity of HLA class I antigens of the donor.[57] For instance, the production of HLA-Bw4 antibodies preferentially occurred in HLA-DRB1*01 and HLA-DRB1*03 positive patients.[58]

Following this work, Spierings and colleagues investigated the role of donor HLA-derived T-helper epitopes in formation of *de novo* DSA *in silico*. To predict T-helper epitopes, an algorithm was developed to determine the number of HLA class II-restricted predicted recognizable HLA epitopes (PIRCHE-II) for each HLA class I antigen mismatch. PIRCHE-II are theoretical epitopes consisting of nine amino acids present on the mismatched HLA class I antigen and absent on all HLA class I antigens of the recipient, which are able to be presented in peptide binding groove of the HLA class II molecules of the recipient.

In a cohort study of non-immunised renal transplant patients, a correlation was observed between a low number PIRCHE-II, and the lack of *de novo* antibody responses against specific HLA class I mismatches.[59] The role of PIRCHE-II in antibody formation was subsequently confirmed in a pregnancy cohort.[60] Noteworthy, these studies indicate a predictive value of PIRCHE-II on the population level, but it does not show any clinical relevance for the individual patient. Furthermore, the PIRCHE-II algorithm likely overestimates the number over PIRCHE-II as it is unlikely that the proteasome indeed processes all these theoretical peptides. Additionally, the functional role of these T-helper epitopes with respect to the activation of $CD4^+$ T cells remains to be proven.

Interestingly, in both transplantation and pregnancy cohorts no correlation between the number of PIRCHE-II and eplet mismatches was found. This might be explained by the fact that the eplet model is restricted to polymorphisms at antibody accessible sites. Furthermore, this model does not take into account the restriction of presentation capacity by HLA antigen towards helper T cells. An alternative, yet not exclusive explanation could be the involvement of noncognate T-helper responses to multiple HLA disparities, in which B cells and T-helpers cell do not necessarily have to recognise the same antigen. The group of Pettigrew showed in murine models of transplantation that B cells specific for an alloantigen can receive help from T-helper cells with another allospecificity. Long-lasting humoral alloimmune responses can be explained by this mechanism as memory T-helper cells recognising one alloantigen can give CD4-independent help to B cells that are specific for another alloantigen.[61] To optimally predict the immunogenicity of an HLA mismatch for an individual patient, both antibody epitopes and T cell epitopes should be considered.

Immunogenicity of epitopes in clinical renal transplantation

The immunogenicity of epitopes in clinical renal transplantation has been extensively studied.[22,46-48,51-53] In these studies, the HLAMatchmaker computer algorithm was used to determine the epitope load of certain HLA mismatches in order to correlate these with

the development of *de novo* DSA. A significant correlation between the number of epitope mismatches between recipient and donor and the chance to develop *de novo* DSA was observed.

Wiebe *et al.* used HLAMatchmaker in a population study and observed no DSA production in recipients with 10 or less HLA-DR eplet mismatches and 17 or less HLA-DQ eplet mismatches.[48] In addition, recipients who were nonadherent to their immunosuppressive medication and received a transplant with an HLA class II eplet load above these thresholds were more likely to develop DSA, acute rejection and graft failure compared to adherent recipients with an epitope load beneath the thresholds, indicating a synergistic effect of nonadherence and HLA class II eplet load.[62] It is important to note that in this study the HLA class II eplet threshold was based on all potential eplets, rather than antibody-verified eplets. Using the same threshold another group found that paediatric recipients of a graft with a DQ eplet load below the threshold were at a low risk to develop both DR and DQ specific *de novo* DSA.[63]

As a possible consequence of the association between the number of HLA class I and II epitope mismatches and development of *de novo* DSA, the number of epitope mismatches appeared to be a predictive parameter for the outcome of graft survival and long-term outcome of renal transplant recipients.[62,64-67] Sapir-Pichhadze *et al.* even described an association between the number of HLA-DR and -DQ eplet mismatches and development of transplant glomerulopathy.[68] The sum of HLA-DR and -DQ eplet mismatches was found to be an independent risk for antibody mediated rejection and transplant glomerulopathy. Besides renal transplants, epitope matching appears also to be beneficial for other types of solid organ transplants. In both lung transplant recipients and heart transplant recipients the number of HLA eplet mismatches correlated with the development of DSA and consequently the outcome of the transplantation.[69,70]

Altogether, these studies indicate the higher the number of HLA class I and II epitope mismatches the higher the risk of developing *de novo* DSA, without yet considering the differential immunogenicity of the individual epitopes.

ANTIGENICITY OF AN ANTIBODY EPITOPE

Reactivity of existing antibodies

So far, we have discussed that the trigger for antibody production by a B cell is the presence of a foreign immunogenic epitope on a mismatched donor HLA molecule. Subsequently, the reactivity pattern of the induced antibody determines the antigenicity of an epitope. Not only the immunogenic epitope, but also other amino acids in its proximity are involved in the actual reactivity of the antibody and formation of the antigen-antibody complex.

Antigen-antibody complexes are formed by three complementary determining regions (CDR) on the variable heavy chain (VH) and three CDRs on the variable light chain (VL)[71] that bind to six contact sites on the immunising HLA molecule, forming an antibody 'footprint' of 700-900 Å on the molecular surface of an HLA molecule. The VH3 binds to the immunogenic epitope and is responsible for the strength and specificity of the antibody reactivity. The remaining CDRs are important for stability and affinity of the antibody binding and will bind to amino acids within the antibody footprint.

As mentioned, HLA molecules can share amino acid configurations and thus also the contacts sites involved in antigen-antibody complexes. This may explain the broad reactivity of antibodies observed in SAB assays when screening sera which not only contain DSA but also non-donor specific antibodies (NDSA).[72,73] All the reactive HLA molecules contain the immunogenic epitope of the mismatched HLA molecule that triggered the antibody response and often one or more critical amino acid configurations important for antibody binding (Figure 1). This immunogenic epitope and/or critical amino acid configurations are absent on the non-reactive HLA molecules. So, the actual reactivity of antibodies depends on both the immunogenic epitope and the other crucial amino acid configurations of the epitope that act as contact sites necessary to form a stable antigen – antibody complex.

Duquesnoy defines the antibody footprint as a structural epitope consisting of 15-22 amino acids. In the centre 2-5 amino acid residues reside of which at least one is non-self, also known as the eplet, or functional epitope, to which the VH3 of antibodies bind, similar to what we described above.[24,25,74]

Based on this antibody footprint, Duquesnoy analysed the broad reactivity observed in SAB assay of various mAbs with the HLAMatchmaker algorithm and proposed different reactivity patterns.[31,32,55] In one reactivity pattern, all the reactive HLA molecules contain the immunogenic eplet that induced the antibody response, which implies that the VH3 site

of antibody can bind to this eplet, while the non-reactive HLA molecules do not have this immunogenic eplet and as a result the VH3 has no binding site on these molecules.[26] In some cases, it appeared that the non-reactive HLA molecules do have the immunogenic eplet to which the VH3 can bind to, but lack additional crucial amino acid configurations within the antibody footprint that are present on the reactive HLA molecules (Figure 1). The need for two configurations to establish an antigen – antibody complex is designated as eplet pairs.[31,32,55]

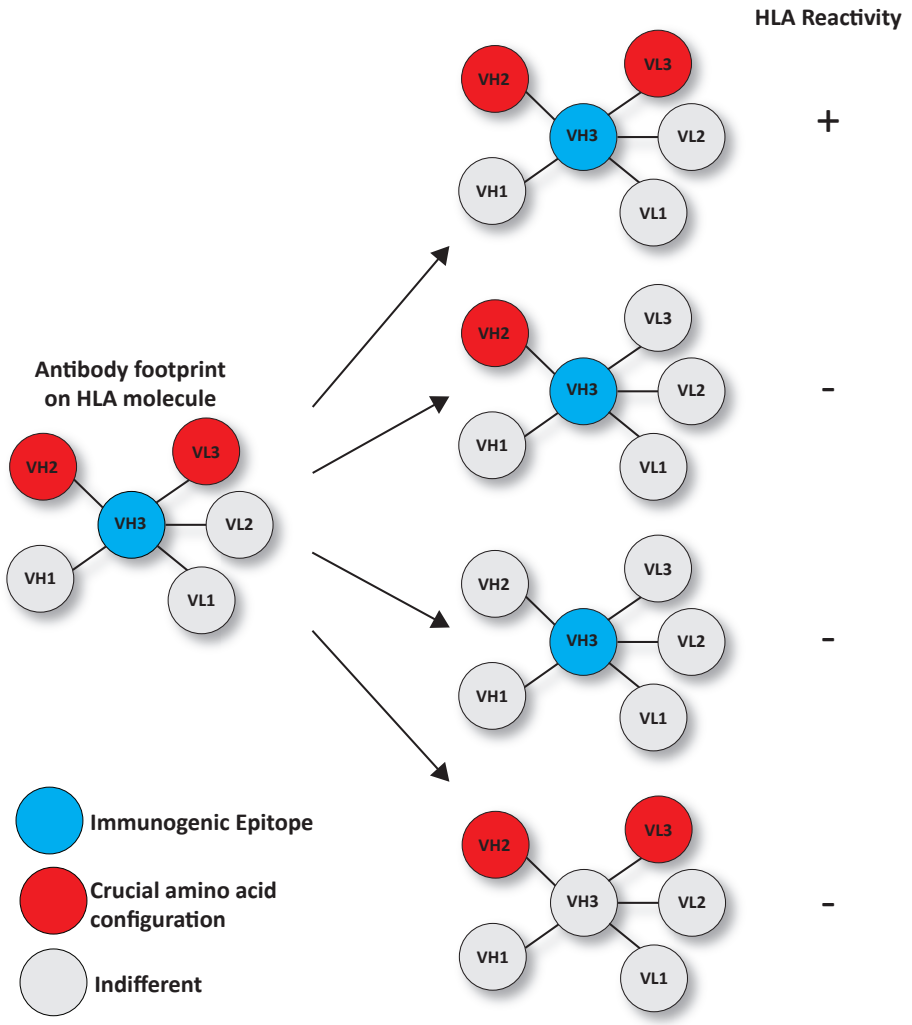

Figure 1. Crucial amino acid configurations determine the reactivity of an HLA antibody. Schematic representation of an antibody footprint present on an HLA molecule. The VH3 of the antibody binds to the immunogenic epitope (blue), while binding of other contact sites of the antibody to crucial amino acid configurations (red) is essential for reactivity. The remaining amino acid configurations within the antibody footprint are indifferent to the reactivity.

Additionally, Duquesnoy *et al.* showed different reactivity patterns detected when screening human HLA-specific mAbs with IgG SAB assays, C1q SAB assays, and complement-dependent cytotoxicity (CDC) assays.[75] Based on these data, they proposed that certain specific polymorphic amino acid configurations of structural epitopes are not only crucial for antibody binding, but also affect the release of free energy, which determines whether complement can be activated.

Complexity of HLA class II reactive antibodies

Defining epitopes of the HLA class II reactive antibodies is in its infancy, as mainly HLA class I-specific human mAbs are currently available. Moreover, epitopes on HLA-DQ and-DP are more complex due to the fact that both α and β chains are polymorphic. Tambur *et al.* have touched on this complexity and the importance of understanding the reactivity of HLA-DQ antibodies.[76,77] Upon analysis of HLA-DQ antibodies, immunogenic eplets are present on either the mismatched DQα or DQβ chain. The antibody footprint can cover an additional crucial polymorphic amino acid configuration on HLA molecule, as indicated in Figure 1, and depending on the location of immunogenic eplet these crucial configurations are located on either one or both DQ chains. As a consequence, it appears that antibodies can react with a self DQβ chain and a non-self DQα chain or vice versa in SAB assays, which complicates the analysis of the reactive antibodies. Tambur emphasises that for assigning the epitope specificity of HLA-DQ antibodies both the DQα and DQβ chains should be considered.[78,79]

Analysis of HLA-DQ antibodies becomes even more complicated when considering the two possible forms of the HLA-DQ antigens, which can be expressed on the cell surface. The first one is the cis-heterodimer, in which the β-chain and α-chain are derived from genes on the same chromosome, the second one is the trans-heterodimer, where the chains are derived from genes on two different chromosomes. Thus, HLA-DQ DSA can theoretically be formed against a DQβ-chain, DQα-chain, or both in either cis- or trans-heterodimer.[80] Extensive analysis of HLA-DP antibodies reactivity is lacking, but the structure of DPβ chain seems less complicated than that of HLA-DQ.

To simplify analysis of HLA class II antibody reactivity the previously mentioned computer model based on physiochemical properties of amino acids can be used.[51,53] With this computer model, high-resolution three-dimensional structural models of HLA class I alleles have been developed to understand the binding of antibodies to an epitope. These models can contribute to the prediction of HLA antigenicity by providing more insight in the antigen-antibody interaction.[81-83] This tool together with the antibody-reactivity patterns, especially

those of mAbs observed in SAB assays, will contribute to a better understanding of the role of both the immunogenic epitope and additional crucial polymorphic amino acid configurations in the antigen-antibody interaction.

USING EPITOPE KNOWLEDGE IN THE CLINIC

Epitope matching

Preventing DSA formation is pivotal in clinical transplantation and therefore it is important to avoid the antibody trigger, which can be any immunogenic epitope. This is especially important for paediatric patients who most likely need more than one transplant in their lifetime. Recently, Kausman *et al.* applied HLA eplet loads for the selection of donors in paediatric kidney transplantation.[84] This group used HLA class I <10 eplets and HLA class II <30 eplets as the threshold for each antigen. In the first year post-transplantation, an excellent early graft function and a low *de novo* DSA incidence was observed. This study indicates that paediatric patients benefit from epitope matching.

Implementation of an epitope matching algorithm for donor selection in renal transplantation may prevent the allocation of kidneys with highly immunogenic epitopes and prevent the induction of DSA. To achieve optimal epitope matching, a proper definition of the actual epitopes and their immunogenicity is of great importance.

Virtual crossmatching

As mentioned, the presence of DSA complicates repeat transplantation, especially for highly sensitised patients as the antibodies will cause a positive crossmatch with the majority of donors. The identification of acceptable and unacceptable HLA antigens on basis of extensive antibody screening[85,86] is currently used to predict beforehand which donor HLA antigens will result in a negative or positive crossmatch[87]: the so called virtual crossmatch.

However, this review shows that epitopes determine antibody reactivity. Therefore, future virtual crossmatching should be based on epitopes (Figure 2). Epitopes present on the non-reactive HLA molecules are instrumental for the definition of acceptable epitopes whereas unacceptable epitopes can be defined on basis of antibody reactivity. For virtual crossmatching, the combination of self-epitopes, acceptable epitopes, and unacceptable epitopes will determine the selection of a suitable donor. Suitable donors will have HLA molecules that consist only of self-epitopes and acceptable epitopes, as these will predict a negative crossmatch. The advantage of the use of acceptable and unacceptable epitopes compared to current strategies of virtual crossmatching, which are based on HLA antigens,

is the fact that one can even predict the antibody reactivity with HLA alleles, that are absent in antibody detection assays such as SAB assay, and thus have never been tested before.

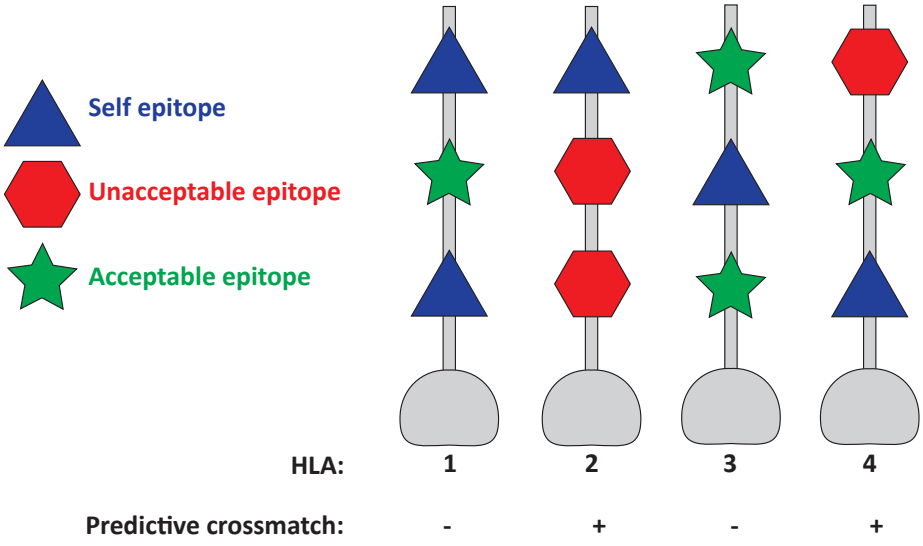

Figure 2. The use of epitope knowledge for virtual crossmatching. Each HLA molecule consists of a set of epitopes. HLA molecules with self-epitopes (blue triangles) and/or acceptable epitopes (green stars) will predict a negative crossmatch, while HLA molecules with unacceptable epitopes (red hexagons) will predict a positive crossmatch.

Highly sensitised patients

As indicated earlier, acceptable epitopes defined by the absence of antibody reactivity can be used to identify donors with acceptable HLA antigen mismatches for highly sensitised patients. The chance of finding a donor with HLA antigens towards which highly sensitized patients have no detectable circulating antibodies is very small. In order to increase this chance, Eurotransplant runs the Acceptable Mismatch (AM) program, in which the sera of highly sensitized patients are screened to determine the acceptable HLA mismatches to which the patient did not form any antibodies.[85,86] This knowledge is used for the selection of compatible donors. A donor kidney with an HLA type consisting of the combination of the patient's own HLA antigens and acceptable HLA mismatches will be mandatorily shipped to that specific highly sensitized patient. Nowadays, the AM program uses HLAMatchmaker to identify additional HLA class I antigens which are likely to be acceptable mismatches due to lack of antibody epitopes. This led to an increased number of HLA class I acceptable mismatches, and subsequently increases the chance of finding a suitable donor.[88-90] Altogether, defining

epitopes absent in antibody-reactivity patterns of sensitized patients will be beneficial for the selection of a donor with HLA molecules consisting of acceptable epitopes.

TOWARDS EPITOPE MATCHING

HLA epitope matching will be beneficial for preventing sensitization, selection of donors for highly sensitised patients, and improvement of transplant outcomes.

To identify the clinically relevant epitopes, cohort studies on antibody induction are extremely useful. However, many studies suffer from limitations, such as restricted numbers of patients included in the analysis. Additionally, the (partial) lack of second field HLA typing (current allocation is often based on intermediate typing of HLA-A, -B, and -DRB1) is an inhibitory factor. To circumvent this problem, HLAMatchmaker contains an algorithm that assigns most likely high-resolution typing based on race of the recipient and/or donor (http://www.epitopes.net/). When second field typing is available for HLA-A, -B, and –DR, the high-resolution typing of the other loci are assigned based on common associations of B-C, and DR-DQ antigens in the given population. While this approach is useful, to move the field forward, high resolution typing is pivotal.[91] While the antibody specificities from SAB assays are on the second-field level, it can appear that an allele-specific antibody is directed against a self-antigen, in case the HLA typing of the patient is only performed at first field level. This is due to differences in polymorphic amino acids that exist between alleles of the same serological antigen. Thus, for understanding the epitope that induced antibodies to a certain HLA mismatch and determining the relevance of these antibodies in transplantation, second field typing of the donor is an absolute requirement.

Most epitope studies used SAB assays for the identification of DSA in sera of recipients. However, analysis of multispecific sera is challenging, especially when more than one immunising event has occurred. In addition, when defining acceptable and unacceptable epitopes based on antibody reactivity observed in SAB analysis, it is essential that the data are interpreted with caution, and that a possible prozone effect is excluded.[92-97]

Several approaches are used to determine the immunogenicity of epitopes, but a systematic study on a large population of patients has not been performed. Large cohort studies combining the different approaches described in this review are essential to discriminate the immunogenic and non-immunogenic epitopes. For the patients carrying rare HLA alleles, it will be difficult to determine immunogenic epitopes, so for the time being these patients will have to take advantage of matching strategies based on theoretical epitopes.

Definition of antibody epitopes is one of the main subjects of the 17th International HLA and Immunogenetics Workshop (IHIWS) that will be held in Palo Alto, CA in September 2017. For this collaborative effort, (high resolution) HLA typing of recipient and donor, screening data from sera of recipient with various SAB assays, and graft outcome data from transplant centres all over the world are collected for various projects, including definition of the immunogenicity of individual epitopes (http://ihiws.org/).

Conclusion

The broad antibody reactivity observed in recipients after transplantation of an HLA antigen mismatched graft can be explained by the presence of mismatched epitopes on the HLA of the donor, which are shared with other HLA alleles. Identification of the immunogenicity of the individual epitopes and avoiding transplantation in the presence of highly immunogenic epitope mismatches will prevent DSA formation. In addition, understanding the complex interaction between the induced antibody and the reactive HLA molecules will contribute to the identification of acceptable mismatches and virtual crossmatching even for highly sensitised patients. It is to be expected that future HLA matching strategies will change from antigen or allele matching towards HLA epitope matching.

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Conflict of interest

The authors have declared no conflicting interests.

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