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Bezstarosti, S.; Kramer, C.S.M.; Claas, F.H.J.; Fijter, J.W. de; Reinders, M.E.J.; Heidt, S.

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Research article

Implementation of molecular matching in transplantation requires further characterization of both immunogenicity and antigenicity of individual HLA epitopes

Suzanne Bezstarosti [a,b], Cynthia S.M. Kramer [a], Frans H.J. Claas [a,c], Johan W. de Fijter [b], Marlies E.J. Reinders [d], Sebastiaan Heidt [a,c,*]

[a] Department of Immunology, Leiden University Medical Center, Leiden, The Netherlands
[b] Department of Internal Medicine (Nephrology), Leiden University Medical Center, Leiden, The Netherlands
[c] Eurotransplant Reference Laboratory, Leiden, The Netherlands
[d] Department of Internal Medicine, Erasmus Medical Center Transplantation Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands

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ABSTRACT

Over the past decade, high HLA epitope mismatch scores have been associated with inferior transplant outcomes using several tools, of which HLAMatchmaker is most well-known. This software uses theoretically defined polymorphic amino acid configurations, called eplets, for HLA compatibility analysis. Although consideration of eplet mismatch loads has potential for immunological risk stratification of transplant patients, the use of eplet matching in organ allocation algorithms is hindered by lacking knowledge of the immunogenicity of individual eplets, and the possibility that single mismatched amino acids, rather than complete eplets, are responsible for HLA antibody induction.

There are several approaches to define eplet immunogenicity, such as antibody verification of individual eplets, and data-driven approaches using large datasets that correlate specific eplet mismatches to donor specific antibody formation or inferior transplant outcomes. Data-driven approaches can also be used to define whether single amino acid mismatches may be more informative than eplet mismatches for predicting HLA antibody induction.

When using epitope knowledge for the assignment of unacceptable antigens, it important to realize that alleles sharing an eplet to which antibodies have formed are not automatically all unacceptable since multiple contact sites determine the binding strength and thus biological function and pathogenicity of an antibody, which may differ between reactive alleles.

While the future looks bright for using HLA epitopes in clinical decision making, major steps need to be taken to make this a clinical reality.

1. Introduction

Antibody-mediated rejection is still a major cause of allograft failure in kidney transplantation despite the use of powerful immunosuppressive drugs. Although HLA matching has significantly improved graft survival, many patients develop *de novo* donor-specific antibodies (dnDSA) against mismatched donor HLA, which are associated with rejection and poor graft survival [1,2]. Refinement of HLA matching strategies could decrease dnDSA formation, but with over 30,000 HLA alleles known, HLA matching on the allele level is not clinically feasible. Interestingly, the high level of polymorphism between these thousands of alleles is explained by only a few hundred antigenic determinants, called epitopes. Accordingly, every HLA allele can be regarded as a unique set of epitopes, while individual epitopes can be shared between different HLA alleles [3]. Due to epitope sharing between alleles, matching on the epitope level could be a feasible strategy to refine HLA matching. Over the years, many studies have demonstrated that molecular mismatch loads are associated with inferior trans-

Abbreviations: dnDSA, *de novo* donor-specific antibodies; EMS, electrostatic mismatch score; HLA, human leukocyte antigen; mAb, monoclonal antibody; SAB, single antigen beads.

* Corresponding author at: Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, The Netherlands.
E-mail address: s.heidt@lumc.nl (S. Heidt).

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plant outcomes. However, since not every molecular mismatch will lead to antibody formation, knowledge of the relative immunogenicity of individual HLA epitopes is necessary before epitope matching can be implemented in clinical transplantation. In this review, the different factors affecting HLA immunogenicity and an overview of studies reporting differential immunogenicity of individual epitopes will be presented.

2. Determinants of HLA immunogenicity

HLA immunogenicity is principally based on mismatched amino acid residues between the donor and recipient HLA. The first studies that investigated immunogenicity of HLA in the context of the recipient's HLA type were able to identify specific donor-recipient HLA antigen combinations that were associated with an increased risk of graft loss in kidney transplantation [4,5]. On the other hand, also permissible HLA antigen mismatches associated with increased graft survival were described [6]. It was hypothesized that some HLA antigen mismatches were permissible due to polymorphic amino acid configurations on the donor HLA that were also present on the recipient, and would thus not be recognized as foreign. The challenge of finding suitable donors for highly sensitized kidney transplant patients led to the development of HLAMatchmaker by Rene Duquesnoy. This program allowed for the comparison of amino acid sequences of donor and recipient HLA alleles to identify mismatched amino acid triplets as potentially immunogenic epitopes [7]. Indeed, triplet mismatches were demonstrated to be associated with alloantibody formation in kidney transplant recipients and pregnancy-immunized women [8]. Further development of HLAMatchmaker resulted in the introduction of the term ''eplet'' to describe polymorphic amino acid residues within a 3.0–3.5 Ångstrom radius, which can be discontinuous, as opposed to the linear amino acid triplets [9], due to the conformational nature of epitopes recognized by the B cell receptor. Since then, many studies have demonstrated the association between high eplet mismatch load and increased risk of dnDSA formation, transplant glomerulopathy, rejection and graft failure in kidney transplantation [10–17]. Eplet mismatch load has also been shown to be an independent predictor for chronic lung allograft dysfunction [18], graft loss in pediatric heart transplantation [19] and dnDSA formation in liver transplantation [20].

Box 1. Epitope vs Eplet

Epitope: The HLA epitope can be described using two definitions; the functional and the structural epitope. The functional epitope determines the specificity of the antibody through its interaction with the complementarity-determining region 3 (CDR3) of the heavy chain of the antibody. The structural epitope comprises all amino acids of the HLA-molecule that are involved in the binding to the antibody paratope and spans a radius of approximately 15 Ångstrom.

Eplet: The definition of an eplet resembles the functional epitope and comprises the minimal amino acid configuration on the HLA-molecule that is needed to induce an antibody response. Involved residues must be within 3 -3.5 Ångstrom.

2.1. Amino acid mismatches and their physiochemical properties

Not every eplet mismatch between donor and recipient will lead to an antibody response, since immunogenicity of HLA not only depends on the number of eplet mismatches, but also on the physiochemical properties of the polymorphic amino acids compared those of the recipient. The interaction between the B cell epitope and the paratope on the B cell receptor is characterized by surface-accessible amino acids that form noncovalent bonds (hydrophobic, van der Waals and hydrogen bonds, and salt bridges), which are regulated by the electrostatic properties of polar and charged amino acid residues [21–23]. Indeed, physiochemical disparity, defined as electrostatic charge and hydrophobicity, between mismatched HLA-A, -B, -DR and -DQ molecules was associated with alloantibody formation in a cohort of highly sensitized kidney transplant recipients [24,25]. Moreover, Kosmoliaptsis et al. demonstrated that substitution of critical amino acids of a Bw6 epitope led to striking changes in the electrostatic pattern of the epitope and resulted in the abrogation of the antibody binding [26]. Although this is primarily confirming that electrostatic properties are of importance for HLA antigenicity (i.e. the binding capacity of the antibody), it is evident that physiochemical properties are important determinants for HLA immunogenicity as well. Accordingly, the Electrostatic Mismatch Score (EMS), developed by the Cambridge group, allows for the comparison of electrostatic potential between donor and recipient HLA. EMS scores of HLA-DR and -DQ were demonstrated to be a predictor of alloantibody formation in patients that experienced kidney graft failure [27] and in kidney transplant recipients with predominantly low immunological risk [14]. However, EMS was not superior to amino acid mismatch or eplet mismatch scores as a predictor for dnDSA formation. Meanwhile, the EMS program has been further developed to take into account the tertiary structure of HLA molecules. This EMS-3D score was shown to be associated with alloantibody formation in women that received an injection with donor lymphocytes from their male partner, and in kidney transplant patients that returned to the waiting list after graft failure [28].

More recently, the HLA Epitope Mismatch Algorithm (HLA-EMMA) was released. This program does not only allow for batch analysis of amino acid mismatches between donors and recipients, but also identifies solvent-accessible amino acids, which are residues that are accessible for the B cell receptor and could therefore potentially interact with the B cell receptor, as well as with antibodies [29]. Since the eplet repertoire has been subject to continuous change, results and threshold values for eplet mismatch load from different studies are difficult to compare. This is not the case for amino acid mismatch analysis, since the amino acid sequences of HLA alleles are fixed entities. Since consideration of all solvent-accessible residues probably results in an overestimation of the clinically relevant amino acid polymorphisms, definition of differential immunogenicity of single amino acid mismatches still is required.

2.2. Availability of T cell help

HLAMatchmaker, EMS and HLA-EMMA are all tools to calculate molecular mismatches regarding epitopes that interact with the B cell receptor (Fig. 1). However, proliferation and differentiation of naïve B cells into memory B cells and plasma cells requires the help of cognate $CD4^+$ helper T cells. In this process, peptides of the donor HLA are presented to the T cell receptor in the context of HLA class II molecules on the B cell [30,31]. Hence, whether a sustainable and class-switched donor-specific antibody response can be formed is dependent on the presence of T cell epitopes that can be presented in the recipient's HLA class II molecules and can be recognized by the T cell receptor. Indeed, the HLA-DR phenotype of the responder has been associated with alloantibody formation against HLA class I mismatches [32,33]. PIRCHE-II (predicted indirectly recognizable HLA epitopes presented by HLA class II) is an *in silico* model that predicts HLA-derived peptides that can be presented in HLA class II [34]. PIRCHE-II scores are moderately correlated with eplet mismatch loads [13,35,36] and have been associated with dnDSA formation and graft failure

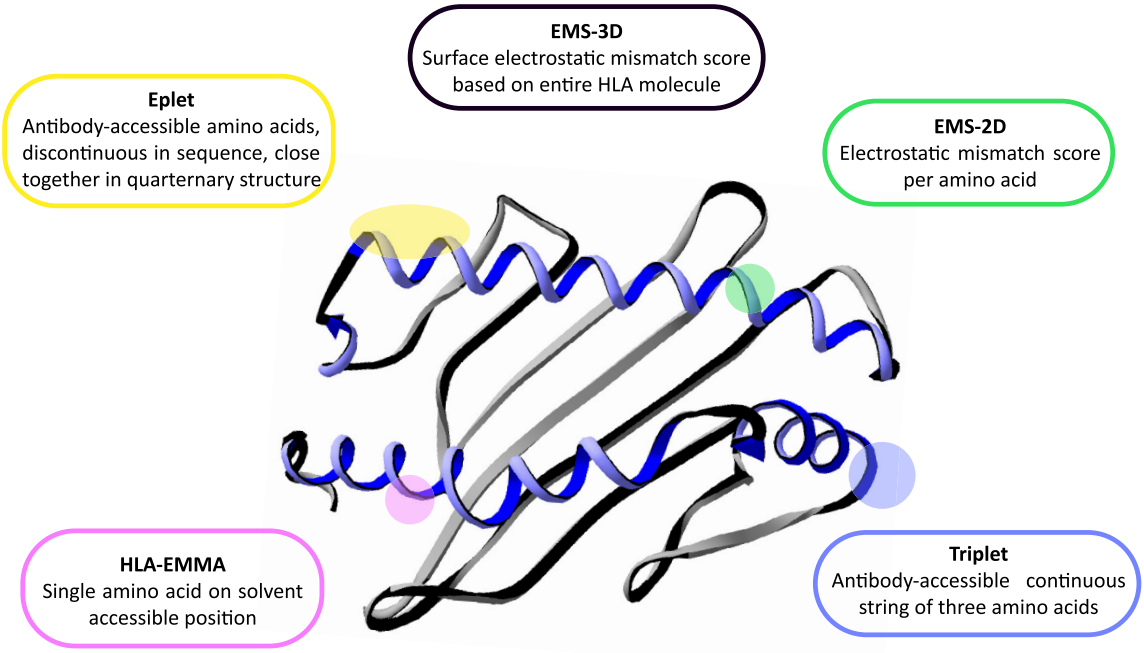


Fig. 1. Current approaches to define HLA molecular mismatches.

in kidney transplantation [13,35,37], and liver transplantation [36,38]. Since there are no data available on which actual peptides will be formed and presented *in vivo*, the T cell epitopes that are predicted by PIRCHE-II are purely theoretical, and probably only a proportion of predicted PIRCHE-II will be of clinical relevance for the individual patient. Availability of T cell help can therefore be regarded as a factor that influences HLA immunogenicity for antibody induction indirectly. Although peptide-binding predictions have been improved [39], they are associated with low specificity [40] and it is unclear how *in silico* predictions relate to *in vivo* immunogenicity in the transplant setting. Therefore, the next section of this review is dedicated to the differential immunogenicity of HLA epitopes interacting with the B cell receptor.

Box 2. Immunogenicity and antigenicity **Immunogenicity:** Immunogenicity of an eplet or functional epitope is the capacity to induce an immune response.

Antigenicity: Antigenicity is the ability of the amino acids making up the structural epitope to be bound by pre-existing antibodies. Therefore, whether a particular eplet-bearing HLA allele is bound by an alloantibody is not only determined by the presence of the eplet, but can also be influenced by amino acids surrounding the eplet, or by the peptide in the peptide-binding groove. Also amino acids that cause a conformational change in the HLA molecule can influence antigenicity, even when they are located outside (but adjacent to) the range of the structural epitope.

3. Differential immunogenicity of HLA epitopes

Several of the aforementioned studies have defined thresholds of eplet mismatch loads above which transplant patients are at risk for inferior outcomes [10,11,35,41,42]. However, the fact that patients can develop dnDSA despite an eplet mismatch load which is below these previously defined thresholds, demonstrates the issue with this approach [17,43]. Clearly, not all epitope mismatches are equally immunogenic, and the association of eplet mismatch load with dnDSA and graft survival merely shows that a higher number of mismatches increases the chance that immunogenic epitopes are present. Furthermore, the determination of eplet mismatch thresholds is not only dependent on the investigated population, but also on the version of HLAMatchmaker that is used for eplet mismatch analysis, since the total number and repertoire of eplets in the different versions of HLAMatchmaker varies. Therefore, although molecular mismatch loads can provide insight for risk stratification of transplant patients, the evaluation of differential immunogenicity of individual HLA epitopes is of critical importance before HLA epitope matching can be implemented in organ allocation algorithms.

3.1. Experimental verification of HLA epitopes

The eplets in the HLAMatchmaker software and the HLA Epitope Registry website have been theoretically defined based on the comparison of amino acid sequences of HLA alleles. Therefore, it is likely that not all of these theoretical eplets will be able to induce alloantibody formation. The HLA Epitope Registry has gathered information on experimental verification of HLA epitopes which has been used to classify eplets as "antibody-verified" [44–47]. Antibody verification is the most fundamental method to assess clinical relevance of individual epitopes, by validating that the epitope can be bound by alloantibodies. Several studies have investigated the subset of antibody-verified eplet mismatches as a risk factor for rejection and graft loss [16,17,20,48]. The antibody verification status of eplets as listed in the HLA Epitope Registry is based on several different methodologies: single antigen beads (SAB) assay reactivity analysis of 1) HLA-specific human monoclonal antibodies (mAbs), 2) adsorbed and eluted antibodies from patient sera, 3) sera from uni- and multiparous women and transplant recipients and 4) murine HLA-specific mAbs [45]. The aim of these approaches is to determine to which amino acid residue or eplet the antibody is directed by identification of amino acids that are solely present on the reactive alleles and are absent on non-reactive and self-alleles of the antibody-producer. Although HLAMatchmaker eplets have been classified as antibody-verified regardless of the method of verification, there

is a substantial discrepancy in the level of evidence between the different approaches. Importantly, sera from immunized patients are generally not suitable for epitope verification, because of the polyclonal nature of the antibody response. Also murine mAbs are not sufficient for eplet verification, since murine mAbs might recognize different HLA epitopes than human alloantibodies. Therefore, the most conclusive method for antibody verification is by human mAb analysis. A number of eplets has been verified using human mAbs derived from human B cell hybridoma's [49–51] and human recombinant mAbs [52]. Additionally, adsorption and elution of human alloantibodies from patient sera using single antigen cell lines has resulted in the antibody verification of a considerable number of eplets [53–57]. Although adsorption and elution using single antigen cell lines or beads does not guarantee that the reactivity pattern in SAB analysis is caused by reactivity against a single epitope, antibody-verification of several eplets that have been verified by adsorption and elution has been confirmed by human mAbs. SAB data analysis can be complicated when multiple uniquely shared amino acids are identified that cannot form an eplet together because the residues are too distant from each other. Additional experiments such as mutation studies or crystallography are then necessary to determine the location of the antibody-antigen interaction [58]. Furthermore, while the aim of experimental verification of epitopes is to determine immunogenicity, it is antigenicity that is measured in SAB assays. In that respect, second field HLA typing data of the antibody producer and immunizer are crucial for determining the amino acids that have induced the antibody-response and for distinguishing the immunogenic amino acid(s) from the amino acid residues that contribute to the binding capacity (antigenicity).

While experimental verification of epitopes using human mAbs and adsorption and elution studies provides the opportunity for detailed analysis of HLA epitopes, this method is time-consuming and restricted by the availability of suitable reagents. Therefore, additional approaches to determine HLA epitope immunogenicity are required.

3.2. Identification of immunogenic HLA epitopes in transplant recipients

Several studies investigating molecular mismatch loads in transplant cohorts have also reported on the immunogenicity of individual eplets. The majority of these studies focus on HLA-DQ as it has become clear that the majority of dnDSA is directed towards HLA-DQ molecules [59]. Wiebe et al. reported three HLA-DR and three HLA-DQB eplets that that were associated with dnDSA, of which four were independent predictors of dnDSA formation in therapy-adherent kidney transplant recipients. Interestingly, the two other eplets were only significantly associated in a subgroup analysis of nonadherent patients, suggesting that immunogenicity of individual epitope mismatches is affected by the use of immunosuppression [10]. A later study in a Japanese cohort of previously unsensitized kidney transplant recipients found that patients with at least one of these highly immunogenic HLA-DQ eplet mismatches had a higher risk for chronic-active antibody-mediated rejection [60]. Two other studies also describe several individual HLA-DQ eplets that are suggested to be highly immunogenic. In a cohort of kidney transplant patients that were randomized to switch from cyclosporine to everolimus at three months post-transplantation, DQ7 was the most frequent target of dnDSA in the everolimus-treated cohort. In a subgroup analysis of the DQ7 mismatched patients, five DQ7 eplets were associated with anti-DQ7 dnDSA while seven others were not [61]. Contrastingly, two of the HLA-DQ eplets that were not associated with dnDSA in this study were the most frequent target of DSA in a cohort of liver transplant recipients [62], showing the need for large datasets to be able to define relative immunogenicity of individual eplets.

In a cohort of cardiothoracic transplant patients, McCaughan et al. observed that the majority of dnDSA formation was directed against eplet $45GE_3$ (this eplet is called 52LL in the 2020 update of the HLA Epitope Registry) on HLA-DQ2 and 45EV/55P on HLA-DQ7 [63]. Interestingly, persistent DSA against DQB1*02:01 only occurred when a mismatched donor DQA1*05 allele was present, while no DSA were formed in case of a DQB1*02:01/DQA1*02 mismatch. Since the DQA1*05 allele was self for several patients, the authors concluded that the DSA could not be directed against DQA1*05 only, and hypothesized that a single amino acid polymorphism on the DQA1*05 allele must be part of the structural epitope, which comprises all amino acids of the HLA-molecule that are involved in the binding to the antibody paratope and spans a radius of approximately 15 Ångstrom. Analysis of this particular residue revealed that this polymorphism caused a 4-fold increase in electrostatic potential which could account for the increased immunogenicity. In a similar fashion, a DQB1*03:01/DQA1*05:01 was defined as ''risk epitope mismatch'', although in several cases dnDSA to DQB1*03:01 occurred in the presence of donor allele DQA1*03, instead of DQA1*05. The two risk epitope mismatches could be validated in a lung transplant cohort as a predictor for dnDSA formation, which warrants further investigation regarding the association of dnDSA towards these risk epitope mismatches with outcomes such as rejection and graft loss. The finding that in this cohort HLA-DQ immunogenicity is affected by the combination of the alpha and beta chain of the molecule emphasizes the complexity of HLA epitope analysis, especially for HLA-DQ.

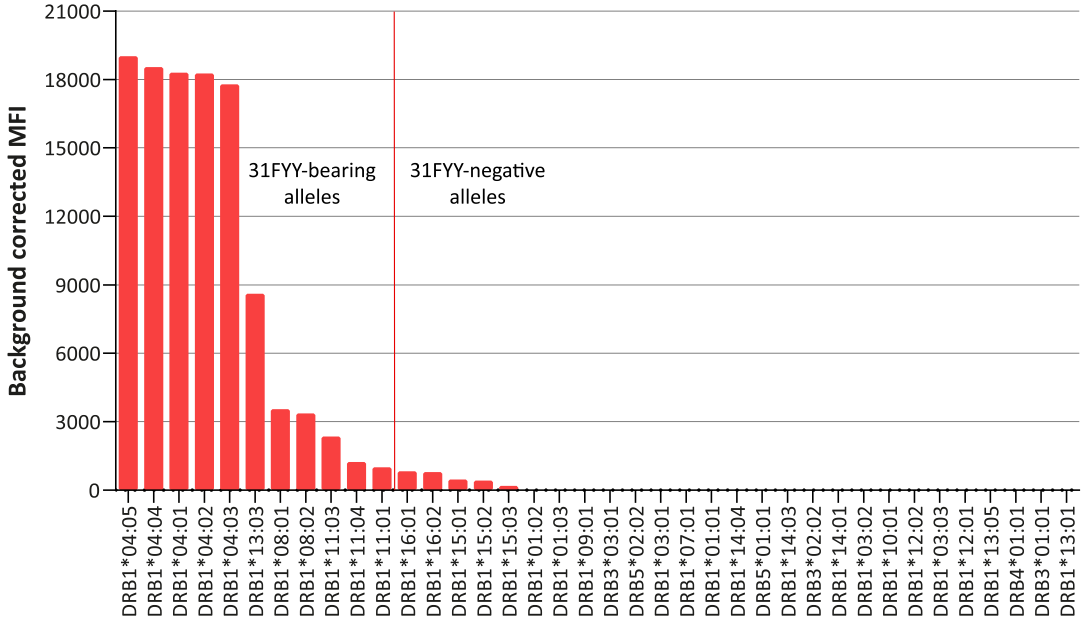
HLA-DQ immunogenicity was also subject of investigation in a concept study by Tambur et al. [43], in which kidney transplant patients that received a graft with 2 HLA-DQ mismatches but developed DSA against only one of these mismatches were analyzed. Since the immunogenic and permissive allele are present in the same patient, this ''2 mismatch, 1 DSA'' approach allows for the elimination of external factors that could affect immunogenicity, such as immunosuppression and comorbidities. Electrostatic mismatch and structural analysis of a number of cases demonstrated that it is preferable to analyze the mismatch of the donor allele in the context of each individual recipient HLA-DQ molecule, because analyses that regard 2 alleles of 1 locus as one entity might disregard specific mismatched amino acid polymorphisms. Furthermore, it was observed that in several cases, the molecular mismatch score was lower for the DSA allele than for the non-DSA allele, affirming that immunogenicity of HLA epitopes is not just a numbers game.

3.3. Immunogenicity of eplets in pregnancy

While the studies discussed in section 3.2 reported on the immunogenicity of a small number of eplets/epitopes, two recent studies have pursued to determine the differential immunogenicity of all antibody-verified HLA class I eplets [64] and the total number of HLA-DQ eplets [65]. In a cohort of pregnancy-immunized women, SAB data from serum collected after delivery was analyzed in HLAMatchmaker to assign child-specific antibodies. Each eplet was assigned an immunogenicity score by dividing the incidence of the eplet mismatch by the incidence of eplet-specific antibodies. A major limitation of this approach is that by testing polyclonal sera in SAB, it is not possible to determine which eplets are truly targeted by alloantibody when multiple overlapping eplets could explain the reactivity pattern. This issue, which is discussed by the authors [65], diminishes the accuracy of the calculated immunogenicity scores. Furthermore, the frequency of particular alleles in the studied population and the number of alleles that share a specific eplet can also introduce bias in immunogenic-

ity scores. This is illustrated by the five most immunogenic HLA class I eplets identified by Hönger et al.; eplet 62GK has the highest immunogenicity score and is shared by three HLA-A2 alleles in the SAB panel. The other four eplets are also shared by HLA-A2, amongst other alleles. Because it is not possible to determine to which eplet the antibody-response is directed, it is possible that the high immunogenicity score of eplet 62GK (which is considered antibody-verified solely based on murine mAb analysis in the HLA Epitope Registry) is in fact caused by antibodies directed to one of the other four eplets present on HLA-A2, which are all antibody-verified by human mAbs or absorption and elution studies. Lastly, since these studies have been performed in individuals without immunosuppressive treatment, it remains to be established whether these results can be extrapolated to transplant patients.

A



B

Allele	BCM	CDC	9	10	11	12	13	16	30	31	32	33	37	47	57	58	67	71
DRB1*04:05	19022		E	Q	V	K	H	H	Y	F	Y	H	Y	Y	S	A	L	R
DRB1*04:04	18536		E	Q	V	K	H	H	Y	F	Y	H	Y	Y	D	A	L	R
DRB1*04:01	18293	pos	E	Q	V	K	H	H	Y	F	Y	H	Y	Y	D	A	L	K
DRB1*04:02	18266		E	Q	V	K	H	H	Y	F	Y	H	Y	Y	D	A	I	E
DRB1*04:03	17788		E	Q	V	K	H	H	Y	F	Y	H	Y	Y	D	A	L	R
DRB1*13:03	8610	pos	E	Y	S	T	S	H	Y	F	Y	N	Y	Y	S	A	I	K
DRB1*08:01	3547	pos	E	Y	S	T	G	Y	Y	F	Y	N	Y	Y	S	A	F	R
DRB1*08:02	3372		E	Y	S	T	G	Y	Y	F	Y	N	Y	Y	D	A	F	R
DRB1*11:03	2351		E	Y	S	T	S	H	Y	F	Y	N	Y	F	D	E	F	E
DRB1*11:04	1233		E	Y	S	T	S	H	Y	F	Y	N	Y	F	D	E	F	R
DRB1*11:01	1006		E	Y	S	T	S	H	Y	F	Y	N	Y	F	D	E	F	R
DRB1*16:01	834	neg	W	Q	P	K	R	H	Y	F	Y	N	S	Y	D	A	F	R
DRB1*16:02	798		W	Q	P	K	R	H	Y	F	Y	N	S	Y	D	A	L	R
DRB1*15:01	467	neg	W	Q	P	K	R	H	Y	F	Y	N	S	F	D	A	I	A
DRB1*15:02	419		W	Q	P	K	R	H	Y	F	Y	N	S	F	D	A	I	A
DRB1*15:03	195		W	Q	P	K	R	H	H	F	Y	N	S	F	D	A	I	A
DRB1*07:01	0		W	Q	G	K	Y	H	L	F	Y	N	F	Y	V	A	I	R
31FYY-negative alleles	≤0																	

Fig. 2. Example of differential antigenicity. (A) Reactivity pattern of human recombinant monoclonal antibody LB_DR4_A in single antigen bead assay. Only HLA-DRB beads are depicted, HLA-DQ and -DP beads were negative. (B) Comparison of the amino acid sequences of positions with residue differences that are within 15 Ångstrom of the eplet defined as 31FY (31F 32Y as listed in the HLA Epitope Registry version 3.0) or 31FYY (31F 32Y 37Y as listed in the HLA Epitope Registry version 2.0 and recently described by Kramer et al. Am J Transplant. 2020;00:1–13). Residue 33H could be involved in the antibody-binding, explaining the stronger interaction of the DRB1*04 alleles with the antibody in the single antigen bead assay. The residue on position 37 appears to play a role in the cytotoxic capacity of the antibody after binding, since DRB1*15 and DRB1*16 have 37S instead of 37Y and are negative in CDC. Additionally, the residue on position 30 might be involved in antibody binding, since DRB1*15:03 has 30H instead of 30Y. Also DRB1*07:01, which is negative in the single antigen bead assay, has 30L instead of 30Y, and 37F instead of 37Y. Residues on position 9–13 are located in the peptide binding groove and are therefore not expected to be involved in the epitope-antibody interaction. HLA-DRB typing of the producer was DRB1*03:01, DRB1*13:01, DRB3*01:01, DRB3*02:02. The immunizing allele was DRB1*04:04. Figure adapted from Kramer et al. Generation and reactivity analysis of human recombinant monoclonal antibodies directed against epitopes on HLA-DR. *Am J Transplant*. 2020;00:1–13. BCM, Background corrected mean fluorescence intensity; CDC, complement-dependent cytotoxicity assay; pos, positive; neg, negative.

3.4. Network-based analysis of eplets

The challenge of the analysis of interrelated eplets has been addressed by Mohammadhassanzadeh et al. in a large study of over 100,000 unsensitized first kidney transplant recipients [66]. In multivariate analyses, they demonstrated significantly increased hazard-ratios for death-censored graft failure for over 200 individual eplets. However, it is possible that only a subset of these eplets are causally related to this outcome, because of clinically relevant eplets simultaneously occurring with less relevant eplets. Therefore, to model the relations between eplets, network analysis was performed, which resulted in the definition of 67 eplet profiles. Most of the eplet profiles that were significantly associated with death-censored graft survival consisted of antibody-verified eplets. However, also a number of single non-antibody-verified eplets were identified to be associated with an increased risk of graft failure. Because of the lack of available high resolution HLA typing data, allele-level typing was imputed from serologic HLA-A, -B and -DRB1 types using an algorithm from National Marrow Donor Program. Although imputation can lead to inaccuracies in eplet mismatch calculations [67,68], imputation of allele-level typing is inevitable for eplet analysis in large retrospective datasets. However, results should be interpreted cautiously, since inaccuracies in eplet mismatch calculation, which might be acceptable in eplet mismatch load analysis in large datasets, could have a considerable impact on analysis of the differential immunogenicity of individual eplets.

Lastly, for both data-driven approaches and studies of smaller patient cohorts, it should be pointed out that multiple versions of HLAMatchmaker have been available with substantial differences in the eplet repertoire both regarding the total number of eplets and the definition (i.e. which residues comprise the eplet). Furthermore, discrepancies between the HLAMatchmaker repertoire and the HLA Epitope Registry have been described [69]. Especially for HLA-DQ, eplet definitions have been subject to change. For instance, in HLAMatchmaker 2.1, which is used in the study of Schawalder et al., eplet 84QL (84Q 86E 87L 89 T 90 T) and 125A are separate eplets. However, in version 3.0, residue 125A has become part of the definition of eplet 84QL. Similarly, 2 of the 5 most immunogenic eplets (52PQ and 85VG) identified in this study have been combined to 1 eplet (52PQ) in version 3.0. These developments should trigger the community to aim at a uniform definition of antibody-verified eplets and a transparent validation of changes in the nomenclature.

4. The role of HLA eplet antigenicity in virtual crossmatching

While the identification of immunogenic eplets is of importance to avoid dnDSA formation in unsensitized patients, especially for pediatric patients that are likely to need a re-transplantation, antigenicity should be considered in the evaluation of unacceptable and acceptable mismatches in (highly) sensitized patients. Antigenicity refers to the ability of the eplet and surrounding amino acids to be bound by pre-existing antibodies and differs from immunogenicity, which is the capacity of an eplet to induce an immune response (Box 2). Historically, the assignment of unacceptable mismatches for highly sensitized patients has been performed based on a serological crossmatch. For unsensitized patients, the virtual crossmatch has now become routine practice for many transplant programs, with the benefit of reducing cold ischemia times [70]. For sensitized patients however, the implementation of virtual crossmatching is more complex. Not only the sharing of eplets by multiple alleles in the SAB assay, which can result in underappreciation of antibody strength [71], but also the differential antigenicity of eplets on different HLA alleles could impede the interpretation of the virtual crossmatch. Specifically, pre-existing DSA against a particular eplet do not necessarily have to recognize all alleles that bear this eplet, due to the other polymorphic residues that play a role in the antigen–antibody interaction. This is illustrated by the analysis of an HLA-specific human mAb in SAB assay where alleles that share a particular eplet can have a wide range of MFI values, for instance ranging from highly positive MFIs exceeding 19,000 to MFI values of 1000 or even lower (Fig. 2) [52]. When it has been ruled out that part of the reactivity can be explained by nonspecific binding, the difference in MFI values results from differential antigenicity of the alleles carrying this eplet. This can be further explored by Luminex analysis that measure C1q-binding, the first component of the classical pathway of complement activation, or complement-dependent cytotoxicity assays. Using these methods, Duquesnoy et al. identified several additional amino acids besides the eplet that could play a role in the binding strength of HLA-specific human mAbs [51]. In the context of (highly) sensitized patient, this means that the presence of pre-existing DSA against a particular eplet does not inevitably mean that every allele that bears this eplet is an unacceptable mismatch. How frequently this phenomenon occurs remains to be elucidated.

5. Conclusion

HLA matching on the epitope level is a potential strategy to refine solid organ allocation in order to decrease formation of dnDSA. Many clinical studies have demonstrated the advantage of epitope matching as opposed to HLA antigen matching on the population level. However, not all epitope or eplet mismatches will be of clinical relevance. Hence, before epitope matching can be implemented in large scale transplantation programs, determination of the relative immunogenicity of individual epitopes is crucial, in order to avoid the denial of suitable organs based on epitope mismatches that are clinically not relevant. To this moment, there have been a number of approaches to define immunogenicity of individual epitopes, each with their strengths and limitations. While experimental verification with human mAbs allows for detailed analysis of epitopes, it is a laborious endeavor that is limited to a small scale. The correlation of dnDSA formation and epitope mismatches in patient cohorts can lead to the identification of immunodominant or risk epitope mismatches, but the heterogeneity of studied populations and the discrepancy between the used eplet definitions restrain the generalizability of the results. Contrastingly, while large-scale data-driven approaches have had to rely on imputed HLA typing for epitope assignment and graft survival as a rather crude primary endpoint, such large datasets can provide valuable knowledge on frequently occurring immunogenic epitopes, as well as less common epitope mismatches. It is therefore evident that these different approaches are complementary and need to be combined through identification of risk epitope mismatches in large patient cohorts, that can be subsequently validated in smaller cohorts with higher data granularity and can be experimentally verified with human mAbs or adsorption and elution studies. Furthermore, generation of a large reference dataset of immunized patients could contribute to investigate the predictive value of the different types and versions of molecular mismatch scores. A major collaboration is currently undertaken under the auspices of the 18th International HLA & Immunogenetics Workshop, which will be concluded in Amsterdam in 2022. Three antigenicity and immunogenicity projects have been dedicated to the definition of immunogenic and non-immunogenic epitopes in order to bring epitope-matching a step closer to clinical reality.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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