Are We Ready for Epitope-Based HLA Matching in Clinical Organ Transplantation?

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Abstract: This overview describes recent developments demonstrating the significance of epitopes in HLA antibody responses and matching for organ transplantation. HLA epitopes are defined by molecular modeling and amino acid comparisons between HLA alleles and the HLAMatchmaker algorithm considers eplets as essential components. Each allele represents a distinct string of eplets and matching is done by aligning donor and recipient strings. Evidence is summarized how mismatched eplet loads affect antibody responses and transplant outcomes. Epitope-based matching has been applied not only to identify acceptable mismatches for sensitized transplant candidates but also to identify more suitably mismatched donors for nonsensitized patients. Three recently proposed theories will further our understanding of the immunogenicity of individual HLA eplets.
It has become apparent that epitope-based matching is superior to antigen matching; we should be ready soon to apply this principle in the clinical transplant setting very soon.

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Ever since the beginning of clinical transplantation HLA has been considered a system of antigens that can induce immune responses leading to allograft rejection and transplant failure. More than 4 decades ago, these antigens were originally defined during international histocompatibility workshops whereby collaborating laboratories identified distinct serum clusters with highly correlated reactivity patterns that permitted arbitrary assignments such as HLA-A1, HLA-B7, and HLA-DR1. Such serum clusters served as references for typing reagents used for the serological identification of HLA antigens. Indeed, HLA matching associations with allograft survivals and documentations of the harmful effects of HLA antibodies on allograft outcome have firmly established the importance of HLA in organ transplantation. However, the general clinical practice has given low priority to HLA antigen matching of transplant donors and there has been a greater emphasis on more effective immunosuppressive drugs to control allograft rejection. Although new immunosuppressive treatments have led to markedly improved long-term graft outcomes, O'Connell[1] pointed out in 2016 during his presidential address to the Transplantation Society that 40% of transplanted organs are lost within 10 years and a high proportion of patients suffer from immunosuppression-related side effects. Moreover, many retransplant candidates become highly alloimmunized to HLA, and it is difficult to find compatible donors. This article expresses the view that HLA antigen-based matching has become obsolete and needs to be replaced by a scientifically more accurate algorithm based on HLA epitopes. As discussed below, Wiebe and Nickerson[2] expressed in their recent review in Transplantation the importance of epitope matching.

What are HLA Epitopes?

Donor-specific HLA antibodies are primary causes of allograft rejection and transplant failure.[3,4] Barbetti et al[5] reported more than 25 years ago that HLA antibodies recognize epitopes that can be defined by amino acid residues, and he pointed out the importance of HLA matching at the epitope level[6-8] and the concept of permissible mismatching to improve long-term graft survival.[9,10]

Modeling of crystalized HLA molecules and amino acid sequence comparisons between HLA alleles have permitted structural descriptions referred to in the HLAMatchmaker algorithm as eplets as essential components of HLA epitopes; several reviews describe molecular models about HLA epitope structures and their reactivity with antibodies[11-15]; the original version used the term triplet.[16] Eplets are small configurations of amino acids in antibody accessible locations on the HLA molecular surface; they are annotated with amino acid sequence numbers and polymorphic residues with standard single-letter codes. Consider for instance, class I sequence position 62. Certain antibodies are specific for the 62GE eplet on A2, B57, and B58 alleles, others recognize 62LQ on A29 alleles and A*43:01 and still others are specific for 62EE on A23 and A24 alleles and A*80:01, and so on.

The International Registry of HLA Epitopes (www.Epregistry.com.br) has records of eplet repertoires for the HLA-A, B, C, DR, DQ, DP, and MICA loci. Eplets are

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theoretical considerations, and each repertoire includes a list of eplets that have been experimentally verified as epitopes specifically reacting with informative antibodies, including human monoclonal antibodies, eluates of antisera absorbed with selected alleles, and antisera with informative reactivity patterns with allele panels. Some studies included testing with mutated HLA alleles. The registry website has an "Epipedia of HLA" file that summarizes the experimental evidence published so far about antibody-verified HLA epitopes 89 of them are HLA-A,B,C alleles, 65 on HLA-DR,DQ, DP alleles, and 22 are on MICA alleles. Such epitopes can be defined by single eplets, whereas others correspond to eplets paired with nearby amino acid configurations, such epitopes are referred to as eplet pairs such as 62QE + 56G and 82LR + 145R.[17,18] Antibody-verified epitopes have been classified as being "confirmed" if the findings appeared conclusive or, with a "provisional" status if the data were considered preliminary; the latter can be upgraded if additional experimental support becomes available.

The HLA Epitope Registry has many eplets that have never been antibody-verified. Is it possible that most of them should be considered as "nonepitopes" because informative antibodies just do not exist? If so, what are the structural and physiochemical criteria that distinguish the epitope and nonepitope nature of eplets? This question is being addressed with a computer modeling program, and the results of this analysis will soon be submitted for publication.

HLA epitope-based matching for transplantation should only consider those epitopes that have been antibody-verified. Obviously, most of them are well known but others are partially understood or even unknown. In its current state, epitope-based matching reminds us of the early days of HLA compatibility when limited understanding was available but was nevertheless, applied in the clinical transplant setting. So the question can be raised: are we ready to explore the implementation of epitope-based matching in organ transplantation?

Many leaders in the field have expressed the opinion that high-resolution HLA typing at the allele level provides more accurate information about donor-recipient compatibility than HLA typing at the antigen level.[19,20] Each HLA allele can be considered as a string of epitopes and matching at the epitope level can be done aligning the eplet string of each donor allele with the eplet strings of recipient alleles. The HLAMatchmaker website (www.epitopes.net) has Excel-based programs for HLA-ABC, HLA-DRDQDP and MICA eplet matching for up to 1000 donor-recipient combinations. They can determine a so-called eplet load of an HLA mismatch, that is, the numbers of mismatched eplets. This depends on the recipient's HLA type with its own repertoire of self-eplets to which no antibodies can be made. Tables 1 and 2 illustrate how the HLA phenotype of the recipient influences the repertoires of mismatched eplets on donor alleles.

The matching program distinguishes between eplets that have been verified experimentally with informative antibodies and "other" eplets that have not been documented as antibody defined. One can expect that the group of antibody-verified eplets would include highly immunogenic epitopes. Table 1 shows for 3 donor HLA-ABC phenotypes how their eplet loads are different among this group of 10 recipient HLA types. With selected cut-offs of 8 antibody-verified eplets, one readily identify mismatches with low eplet loads. This analysis shows also the loads of "other" eplets, but of course, more studies are needed to determine if or not they should be considered clinically relevant. Nevertheless, these findings illustrate how the HLA type of the recipient influences the eplet load of a mismatch.

Table 2 shows for 2 recipients the DR and DQ eplet loads corresponding to the traditionally used DR specificities that have been converted to common haplotypes of DRB1, DRB3/4/5, DQA, and DQB alleles (HLA-DP is not included in this analysis). For these 2 recipients, there are considerable differences between the antibody-verified eplet loads of these haplotypes. Mismatches with high eplet loads are readily identified. Interestingly, DR antigens assigned as self may have mismatched eplets on corresponding haplotypes. Table 2 considers also "other" DR and DQ eplets but there is no experimental evidence which ones if any are antibody-verified.

Effect of Eplet-Based Matching on HLA Antibody Responses and Organ Transplant Outcome

Table 3 summarizes reports showing that eplet loads of class I or class II HLA mismatches correlate with HLA antibody responses after transplantation or induced during pregnancy.[21-30] These findings provide evidence that HLA class I and class II mismatches with low eplet loads are less likely to induce antibody responses.

About 25 years ago, Terasaki's group[7,31,32] reported the influence of HLA epitope mismatching on kidney transplant survival. After the introduction of HLAMatchmaker more than 20 investigations at transplant centers worldwide have suggested that HLA epitope matching is associated with better transplant outcome (Table 4). The first analysis done in 2003 with the United States–based United Network for Organ Sharing (UNOS) and Eurotransplant kidney transplant databases showed that for DR-compatible kidneys the HLA-A,B mismatches with low triplet loads have the similar graft survival rates as the zero HLA-A,B antigen mismatches.[33] In contrast, a study by Laux[34] at the Collaborative Transplant Database suggested no significant association between triplet matching and kidney graft survival but another look at these data clearly revealed similar 5-year graft survivals for the zero-antigen mismatches and the groups with zero or few mismatched triplets.[35] Laux and co-workers[36] have reported that mismatching for DPB at the epitope level is associated with lower kidney transplant survival. The studies summarized in Table 4 demonstrate that HLA epitope mismatching is associated with allograft outcome not only of kidney transplants but also of cornea,[37,38] heart,[39,40] heart valve,[41] lung,[42] and pancreas transplants.[43] Recent studies have suggested that matching for class II eplets encoded by HLA-DR, HLA-DQ, and HLA-DP is also associated with better transplant outcome.[42,44-49]

Although the reports in Table 4 are mostly based on studies including retrospective analyses without multivariate modelling and small case series, these findings provide accumulating evidence suggesting that eplet matching outperforms the traditional HLA antigen-based matching and offers new opportunities to minimize the risk of de novo HLA donor-specific antibodies and enhance transplant success. Other review articles point also out the importance of HLA epitopes in organ transplantation.[50-53]

TABLE 1.

Three examples of HLA-ABC eplet loads for 10 recipient HLA phenotypes

Recipient						Donor 1:	Antibody-verified eplets A*02:03,*03:01;	Other ABC eplets B*53:01,*55:01;	All ABC eplets C*04:01,*05:01
1	A*01:01	A*03:01	B*07:02	B*08:01	C*07:01	C*07:02	16	13	29
2	A*01:01	A*11:01	B*07:02	B*40:01	C*03:03	C*07:02	14	11	25
3	A*02:01	A*32:01	B*35:01	B*56:01	C*04:01	C*12:03	5	7	12
4	A*03:01	A*26:01	B*35:01	B*39:01	C*04:01	C*12:03	12	6	18
5	A*01:01	A*02:01	B*35:01	B*56:01	C*04:01	C*14:01	6	5	11
6	A*30:01	A*68:01	B*51:01	B*57:01	C*08:01	C*07:02	11	7	18
7	A*26*01	A*32:01	B*38:01	B*67:01	C*07:02	C*15:01	14	11	25
8	A*29*02	A*31:01	B*08:01	B*45:01	C*06:02	C*07:01	18	11	29
9	A*01:01	A*24:02	B*51:01	B*35:01	C*01:02	C*04:01	12	9	21
10	A*02:01	A*24:03	B*07:02	B*44:03	C*02:02	C*07:01	8	6	14
						Donor 2:	A*31:01,*68:02;	B*40:02,*58:01;	C*03:01*03:02
1	A*01:01	A*03:01	B*07:02	B*08:01	C*07:01	C*07:02	17	14	31
2	A*01:01	A*11:01	B*07:02	B*40:01	C*03:03	C*07:02	11	10	21
3	A*02:01	A*32:01	B*35:01	B*56:01	C*04:01	C*12:03	7	9	16
4	A*03:01	A*26:01	B*35:01	B*39:01	C*04:01	C*12:03	12	7	19
5	A*01:01	A*02:01	B*35:01	B*56:01	C*04:01	C*14:01	11	9	20
6	A*30:01	A*68:01	B*51:01	B*57:01	C*08:01	C*07:02	7	7	14
7	A*26*01	A*32:01	B*38:01	B*67:01	C*07:02	C*15:01	13	10	23
8	A*29*02	A*31:01	B*08:01	B*45:01	C*06:02	C*07:01	14	8	22
9	A*01:01	A*24:02	B*51:01	B*35:01	C*01:02	C*04:01	11	12	23
10	A*02:01	A*24:03	B*07:02	B*44:03	C*02:02	C*07:01	8	3	11
						Donor 3:	A*26:01,*31:01;	B*39:01.*40:02;	C*03:03,*14:01
1	A*01:01	A*03:01	B*07:02	B*08:01	C*07:01	C*07:02	13	11	24
2	A*01:01	A*11:01	B*07:02	B*40:01	C*03:03	C*07:02	6	7	13
3	A*02:01	A*32:01	B*35:01	B*56:01	C*04:01	C*12:03	11	5	16
4	A*03:01	A*26:01	B*35:01	B*39:01	C*04:01	C*12:03	5	3	8
5	A*01:01	A*02:01	B*35:01	B*56:01	C*04:01	C*14:01	11	5	16
6	A*30:01	A*68:01	B*51:01	B*57:01	C*08:01	C*07:02	12	6	18
7	A*26*01	A*32:01	B*38:01	B*67:01	C*07:02	C*15:01	8	6	14
8	A*29*02	A*31:01	B*08:01	B*45:01	C*06:02	C*07:01	6	4	10
9	A*01:01	A*24:02	B*51:01	B*35:01	C*01:02	C*04:01	10	9	19
10	A*02:01	A*24:03	B*07:02	B*44:03	C*02:02	C*07:01	11	2	13

Application of the Eplet Load Concept in the Clinical Transplant Setting

Wiebe and Nickerson[2] published in a recent issue of Transplantation a review about the strategic use of eplet matching to improve allograft outcomes. Donor-recipient HLA mismatching involves eplets responsible for de novo development of antibodies that cause graft rejection and transplant failure. In this context, an HLA eplet analysis can be considered as a strategy to permit safe immunosuppression minimization to improve patient outcomes. This can be done through an improved organ allocation scheme that favors donor-recipient pairs with low HLA eplet mismatch loads or avoiding highly immunogenic eplets. It involves a personalized immunosuppression to the lowest level possible to avoid metabolic side effects, risks for infection, and malignancy while holding the alloimmune system in check.[2] Their review focused on DR and DQ eplet mismatch loads. For 36% of a cohort of transplant cases, these loads were below the de novo donor-specific antibody development thereby suggesting that a significant proportion of transplant recipients could benefit from this matching approach.

Two transplant centers have reported the application of the eplet load concept as a prospective permissible mismatching strategy for nonsensitized patients as discussed in a recent editorial.[54] The Midwest Transplant Network (Kansas, USA) is using this histocompatibility paradigm for pediatric kidney transplantation whereby donors are selected on the basis of DR and DQ eplet mismatching.[55] Two years ago, the pediatric transplant program in Melbourne, Australia, began to apply an eplet-based Kidney Allocation System and thus far allograft survivals are excellent.[56] Depending on the HLA type of the recipient, mismatched HLA alleles have different numbers of nonself eplets and after establishing threshold values, it is possible to exclude potential donors with high eplet loads. The impact of such exclusions on donor availability can be quantitated as a percentage determined in a similar way as the calculated panel-reactive antibody (PRA) for sensitized patients. One can expect that lower threshold values for eplet loads will

TABLE 2.

Two examples of HLA-DR,DQ eplet loads of common DRB1/3/4/5, DQB1, DQA1 haplotypes of serologically defined DR antigen mismatches

				Recipient 1:	DRB1*03:02,*15:01;	DRB3*01:01, 5*01:01;		DQB1*04:02,*05:02;	DQA1*01:02,*04:01	
					Antibody-verified	Antibody-verified	Antibody-verified	Other	Other	All
Mismatch	DRB1	DRB3/4/5	DQB1	DQA1	DRDQ Eplets	DR Eplets	DQ Eplets	DR Eplets	DQ Eplets	DRDQ Eplets
DR1	DRB1*01:01	—	DQB1*05:01	DQA1*01:01	4	3	1	1	0	5
DR4	DRB1*04:01	DRB4*01:01	DQB1*03:01	DQA1*03:02	17	11	6	6	8	31
DR7	DRB1*07:01	DRB4*01:01	DQB1*02:02	DQA1*02:01	13	8	5	8	6	27
DR8	DRB1*08:01	—	DQB1*04:02	DQA1*04:01	2	2	0	3	0	5
DR9	DRB1*09:01	DRB4*01:01	DQB1*03:03	DQA1*03:02	13	8	5	7	7	27
DR10	DRB1*10:01	—	DQB1*05:01	DQA1*01:01	5	4	1	3	0	8
DR11	DRB1*11:01	DRB3*02:02	DQB1*03:01	DQA1*05:01	7	2	5	3	7	17
DR12	DRB1*12:01	DRB3*02:02	DQB1*03:01	DQA1*05:01	6	1	5	5	7	18
DR13	DRB1*13:01	DRB3*01:01	DQB1*06:03	DQA1*01:03	1	0	1	1	8	10
DR14	DRB1*14:01	DRB3*02:02	DQB1*05:03	DQA1*01:04	0	0	0	9	3	12
DR15(self)	DRB1*15:01	DRB5*01:01	DQB1*06:02	DQA1*01:02	1	0	1	0	5	6
DR16	DRB1*16:01	DRB5*02:02	DQB1*05:03	DQA1*01:02	0	0	0	4	1	5
DR17	DRB1*17:01	DRB3*01:01	DQB1*02:01	DQA1*05:01	6	0	6	0	5	11
DR18 (self)	DRB1*18:01	DRB3*01:01	DQB1*04:02	DQA1*04:01	0	0	0	0	0	0
				Recipient 2:	DRB1*07:01,*13:03;	DRB3*01:01,4*01:01;		DQB1*02:01,*03:01;	DQA1*01:03,*05:01	
DR1	DRB1*01:01	—	DQB1*05:01	DQA1*01:01	15	4	11	2	7	24
DR4	DRB1*04:01	DRB4*01:01	DQB1*03:01	DQA1*03:02	7	3	4	0	4	11
DR7(self)	DRB1*07:01	DRB4*01:01	DQB1*02:02	DQA1*02:01	2	0	2	0	3	5
DR8	DRB1*08:01	—	DQB1*04:02	DQA1*04:01	8	1	7	2	7	17
DR9	DRB1*09:01	DRB4*01:01	DQB1*03:03	DQA1*03:02	6	1	5	4	5	15
DR10	DRB1*10:01	—	DQB1*05:01	DQA1*01:01	14	3	11	0	7	21
DR11	DRB1*11:01	DRB3*02:02	DQB1*03:01	DQA1*05:01	2	2	0	2	0	4
DR12	DRB1*12:01	DRB3*02:02	DQB1*03:01	DQA1*05:01	2	2	0	3	0	5
DR13(self)	DRB1*13:01	DRB3*01:01	DQB1*06:03	DQA1*01:03	7	1	6	2	3	12
DR14	DRB1*14:01	DRB3*02:02	DQB1*05:03	DQA1*01:04	10	0	10	3	9	22
DR15	DRB1*15:01	DRB5*01:01	DQB1*06:02	DQA1*01:02	12	6	6	6	7	25
DR16	DRB1*16:01	DRB5*02:02	DQB1*05:03	DQA1*01:02	15	5	10	10	7	32
DR17	DRB1*17:01	DRB3*01:01	DQB1*02:01	DQA1*05:01	1	1	0	2	0	3
DR18	DRB1*18:01	DRB3*01:01	DQB1*04:02	DQA1*04:01	8	1	7	2	7	17

TABLE 3.

Eplet loads correlate with HLA antibody responses

Year	Investigators	Reported observation	Reference
2004	Dankers Claas (Leiden, The Netherlands)	Mismatched triplet numbers correlate with humoral sensitization after kidney transplantation or induced during pregnancy	21
2006	Goodman Taylor (Cambridge, UK)	Correlation between the number of mismatched epitopes (triplets) and the presence of HLA antibodies detected in Luminex assays with single class I alleles	22
2008	Duquesnoy Marrari (Pittsburgh, PA)	DRB and DQ antibody responses after kidney transplantation correlate with eplet loads	23
2009	Kosmoliaptsis Taylor (Cambridge, UK)	HLA class I alloantigen immunogenicity is predicted by the number and physiochemical properties of amino acid polymorphisms	24
2011	Kosmoliaptsis Taylor (Cambridge, UK)	HLA class DR and DQ immunogenicity is predicted the number and physiochemical properties of amino acid polymorphisms	25
2011	Duquesnoy Marrari (Pittsburgh, PA)	More HLA-C antibody responses by transplant patients who have been exposed to greater HLA-C eplet loads	26
2013	Schaub Honger (Basel, Switzerland)	Number of mismatched HLA-ABC eplets strongly correlates with the rate of child-specific class I sensitization	27
2013	Wiebe Nickerson (Winnipeg, Canada)	Class II HLA epitope matching. A strategy to minimize de novo donor-specific antibody development and improve outcomes	28
2014	Kosmoliaptsis Taylor (Cambridge, UK)	Structural and electrostatic analysis of HLA B-cell epitopes: inference on immunogenicity and prediction of humoral alloresponses	29

decrease HLA antibody responses and enhance transplant success but they will also diminish access to the donor pool. From a practical viewpoint, the selection of a threshold value should consider a balance between the feasibility and the success of a transplant.

Even without prospective eplet-based permissible mismatching, readily obtainable information about the eplet load concept can be used in posttransplant management as a new risk predictor for de novo HLA antibody formation and humoral rejection.[54]

TABLE 4.

HLA epitope matching effect in clinical organ transplantation

Year	Investigators	Reported observation	Reference
2003	Takemoto (UNOS) Claas (Eurotransplant)	Matching at the HLA-A,B triplet level correlates with kidney transplant survival	33
2004	Laux Opelz (Heidelberg, Germany)	Epitope-based HLA-DPB matching affects cadaver kidney re-transplantation	36
2004	Boehringer (Freiburg, Germany)	HLA-AB triplet matching effect on rejection-free survival of corneal transplants	37
2006	Haririan (Detroit, MI)	HLA epitope matching and graft outcome in African-American renal transplant recipients	57
2008	Perasaari (Helsinki, Finland)	Eplet mismatch effect on rejection and coronary artery disease of heart transplant	39
2009	Thaunat Touraine (Lyon, France)	Epitope sharing and chronic humoral rejection mediated by HLA-DP alloantibodies	45
2010	Boehringer (Freiburg, Germany)	HLAMatchmaker matching and operational postkeratoplasty graft tolerance	38
2010	Silva (Santiago, Chile)	HLAMatchmaker-based compatibility predicts graft survival and HLA antibodies.	58
2012	Kneib von Glehn (Curitiba, Brazil)	Eplets and HLA antibodies after implantation of human heart valve allografts	41
2014	Tambur (Chicago, IL)	HLA-DQ epitope specificities of donor-specific antibodies after renal transplantation	44
2014	Wiebe Nickerson (Winnipeg, Canada)	Acceptable mismatching at the class II epitope level: The Canadian experience	46
2014	Zhang (Wenzhou, China)	Application of HLAMatchmaker and eplet matching in renal transplantation	59
2014	Mierzejewska Stepkowski (Toledo, OH)	HLA-DP eplet effect on acute antibody-mediated rejection of a 3rd kidney transplant	60
2014	Mongkolsuk (Bangkok, Thailand)	Shared eplet stimulates antibody-mediated rejection in kidney transplant recipient	61
2014	Bosch Muro (Murcia, Spain)	HLA-C eplet antibodies and irreversible rejection in kidney transplantation	62
2015	Sullivan Warner (Seattle, WA)	HLA epitope mismatching and long-term graft loss in pediatric heart transplant recipients	40
2015	Sapir-Pichhadze (Toronto, Canada)	HLA-DR and -DQ eplet mismatches and transplant glomerulopathy	63
2015	Wiebe Nickerson (Winnipeg, Canada)	Class II HLA eplet mismatch and nonadherence in acute rejection and graft survival	64
2015	Singh Colombe (Philadelphia, PA)	DQ eplet mismatch effects on sensitization trends after renal allograft failure	49
2016	D'Orsogna Holdsworth (Melbourne, Australia)	Epitopes and providing better matched donors through kidney paired donation	65
2016	Sypek Kausman (Melbourne, Australia)	Epitopes and pediatric renal transplantation in the paired exchange program.	66
2016	Pouliquen Thaunat (Lyon, France)	Eplet mismatch numbers anti-donor HLA antibodies after pancreatic islet grafting	43
2016	Walton Westal (Melbourne, Australia)	DRB and DQ eplet mismatching effect on lung transplant failure	42
2016	Kosmoliaptsis Taylor (Cambridge, UK)	Amino acid sequence and physicochemical disparity effects on alloantibodies after renal transplant failure	67
2016	Wiebe Nickerson (Winnipeg, Canada)	Strategic use of epitope matching to improve graft outcomes	2
2016	Milongo Kamar (Toulouse, France)	Donor HLA epitope-specific antibodies isolated from rejected renal allografts	68

HLA Epitopes and Acceptable Mismatches for Sensitized Transplant Patients

Many transplant candidates have serum HLA antibodies that lead the exclusion of donors with unacceptable mismatches. Especially for highly sensitized patients with greater than 95% PRA, it is difficult to find a compatible donor. The antibody analysis programs on the HLAMatchmaker website www.epitopes.net can be used for epitope specificity determinations of HLA antibodies in sera from sensitized patients and the identification of potential donors with acceptable HLA mismatches. This approach shortens the waiting time for a suitable kidney donor and leads to excellent graft survivals comparable to those seen with nonsensitized recipients.[22,69-71] Eurotransplant has incorporated HLAMatchmaker in the Acceptable Mismatch program to identify donors for highly sensitized patients[71-74] and a similar program (Eurostam) has been investigated for implementation in the European Union (Frans Claas, personal communication). Other investigators have also reported the usefulness of HLAMatchmaker in the selection of suitable transplant donors for sensitized patients.[22,75-77]

A computer modeling program about the benefits and costs analysis of implementing a HLAMatchmaker-based acceptable mismatch program in Australia has suggested improved transplant access, reduced waiting time, overall lifetime gain, and financial savings for highly sensitized patients.[50] An HLAMatchmaker-based program to enhance kidney transplantation has also been proposed for highly sensitized patients in Brazil.[51]

Road Toward Implementation of Epitope-Based Matching in Clinical Transplantation

UNOS has recently implemented a new Kidney Allocation System that has increased the transplantation rates for highly sensitized patients.[52,53] A complicated matching algorithm for donors is based on broad and split antigens, so-called antigen equivalences and selected 4-digit alleles, and besides the traditionally used HLA-A, HLA-B, and HLA-DR, it now includes HLA-C, HLA-DQA, HLA-DQB, and HLA-DPB. For each locus, the UNOS database has a WaitList[SM] entry page to record unacceptable mismatches selected from a list of antigens and alleles. Although it seems much easier to use just a list of high-resolution alleles used in serum screening for HLA antibodies, would it be better scientifically to record HLA epitopes associated with antibody specificity as unacceptable mismatches?[78] This approach is consistent with the accepted concept that HLA antibodies recognize epitopes, and it should be noted that most sera including those for highly sensitized patients have antibody specificities to limited repertoires of epitopes.[79-83]

Moreover, the listing of unacceptable mismatches is limited to alleles that are used in the antibody screening assays. For instance, most antibody testing kits have panels with fewer than 100 alleles although many thousands additional alleles have been identified and the list is still growing. Given the increasing racial and ethnic diversity of the general population, one would expect more frequent occurrences of nonpanel HLA alleles in transplant donors. How does one determine the mismatch acceptability of an untested donor allele? Even more, there is often enough a dilemma whereby 2 or more panel alleles corresponding to a same HLA antigen react differently with patient's serum.

An antibody analysis focused on HLA epitopes can readily solve this problem. Table 5 illustrates how information about epitope specificities of recipient antibodies can differentiate between mismatch acceptability and unacceptability of HLA alleles. Two well-defined epitopes have been selected for representative groups of HLA-A, HLA-B, HLA-DRB1 and HLA-DQB1 alleles. Each group has 1 or more alleles generally used in antibody testing assays, they are marked with asterisks. Such panel alleles give positive reactions if they carry

TABLE 5.

Mismatch acceptability examples for recipients with epitope-specific antibodies

Epitope:	82LR	166DG		65QIA	163EW		16H	67LQ + 60Y		56PV	85VA
A*24:02[a]	Positive	Positive	B*07:02[a]	Positive	Positive	DRB1*14:01[a]	Positive	Negative	DQB1*06:01[a]	Negative	Positive
A*24:03*	Positive	Negative	B*07:03	Acc	UnAcc	DRB1*14:02[a]	Positive	Positive	DQB1*06:02[a]	Negative	Positive
A*24:04	Acc	UnAcc	B*07:04	UnAcc	UnAcc	DRB1*14:54[a]	Positive	Negative	DQB1*06:03[a]	Negative	Positive
A*24:05	UnAcc	UnAcc	B*07:05	UnAcc	UnAcc	DRB1*14:03	UnAcc	Acc	DQB1*06:04[a]	Positive	Negative
A*24:06	UnAcc	UnAcc	B*07:06	UnAcc	UnAcc	DRB1*14:04	Acc	Acc	DQB1*06:09[a]	Positive	Negative
A*24:07	UnAcc	UnAcc	B*07:07	UnAcc	UnAcc	DRB1*14:05	Acc	Acc	DQB1*06:05	UnAcc	Acc
A*24:08	UnAcc	UnAcc	B*07:08	Acc	UnAcc	DRB1*14:06	UnAcc	UnAcc	DQB1*06:06	UnAcc	Acc
A*24:10	UnAcc	Acc	B*07:09	UnAcc	UnAcc	DRB1*14:07	UnAcc	Acc	DQB1*06:07	Acc	Acc
A*24:13	UnAcc	UnAcc	B*07:10	UnAcc	UnAcc	DRB1*14:08	UnAcc	Acc	DQB1*06:08	UnAcc	UnAcc
A*24:14	UnAcc	UnAcc	B*07:12	UnAcc	UnAcc	DRB1*14:10	UnAcc	Acc	DQB1*06:10	Acc	UnAcc
A*24:15	UnAcc	UnAcc	B*07:13	Acc	UnAcc	DRB1*14:11	Acc	Acc	DQB1*06:11	Acc	UnAcc
A*24:17	UnAcc	UnAcc	B*07:14	UnAcc	UnAcc	DRB1*14:12	UnAcc	Acc	DQB1*06:12	UnAcc	Acc
A*24:18	UnAcc	Acc	B*07:16	Acc	UnAcc	DRB1*14:15	Acc	Acc	DQB1*06:13	UnAcc	UnAcc
A*24:20	UnAcc	UnAcc	B*07:18	UnAcc	UnAcc	DRB1*14:16	UnAcc	Acc	DQB1*06:14	Acc	UnAcc
A*24:22	UnAcc	Acc	B*07:19	UnAcc	Acc	DRB1*14:17	UnAcc	UnAcc	DQB1*06:15	Acc	Acc
A*24:23	UnAcc	Acc	B*07:20	UnAcc	Acc	DRB1*14:18	UnAcc	Acc	DQB1*06:16	Acc	UnAcc
A*24:25	UnAcc	UnAcc	B*07:22	UnAcc	UnAcc	DRB1*14:19	UnAcc	UnAcc	DQB1*06:17	UnAcc	Acc
A*24:26	UnAcc	UnAcc	B*07:23	UnAcc	UnAcc	DRB1*14:20	UnAcc	UnAcc	DQB1*06:18	UnAcc	UnAcc
A*24:28	Acc	UnAcc	B*07:24	UnAcc	Acc	DRB1*14:24	UnAcc	Acc	DQB1*06:19	Acc	UnAcc

[a] Alleles used in single allele panels used for antibody testing; positive and negative indicates their reactivity with an epitope-specific antibody.
Acc, is acceptable; UnAcc, unacceptable mismatch.

the epitope specifically recognized by antibody, and this means that they carry an unacceptable epitope mismatch. The primary purpose of Table 5 is to demonstrate how epitope specificity information can determine the mismatch acceptability of HLA alleles not used in antibody assays.

Let us consider the 2 eplets selected for A*24 alleles. Antibodies specific for the Bw4-related eplet 82LR react with A*24:02 and A*24:03. Most untested A*24 alleles carry 82LR, and they can be considered as unacceptable mismatches. In contrast, A*24:04 and A*24:28 lack 82LR, and they would be acceptable mismatches for recipients with 82LR antibodies.

The 166DG eplet is shared between A*01:01, A*23:01, A*24:02, A*80:01, and B*15:12 in the antibody screening panel and 166DG-specific antibody reactivity means that A*24:02 is unacceptable but the nonreactive A*24:03 is an acceptable mismatch. Mismatch acceptability of untested A*24 alleles can be readily determined for recipients with 166DG-specific antibodies.

Table 5 has more examples showing how the epitope specificities of recipient antibodies can distinguish between acceptable and unacceptable mismatches among untested alleles encoded by other HLA loci. Many panels have just 1 allele B*07:02, and the mismatch acceptability of the untested B*07 alleles can be assessed from epitope specificities. The DRB example has 3 DRB1*14 alleles, and the DQB example has 5 DQB1*06 alleles in the panel, and although there are different reactivity patterns for the epitope-specific antibodies, one can readily predict the mismatch acceptability of the untested alleles.

Table 5 shows 7 epitopes that can be defined by single eplets but it should be noted that some sera have antibodies specific for epitopes defined by eplet pairs. An example is the antibody-verified 67LQ + 60Y pair that is on the reactive DRB1*14:02 but not on the nonreactive DRB1*14:01 and DRB1*14:54. This epitope is on 4 untested DRB1*14 alleles, all of them can be considered unacceptable mismatches.

For HLA-sensitized transplant candidates, the recording of small numbers of unacceptable epitopes (eplets or eplet pairs) seems more practical than entering long lists of reactive alleles/antigens. This approach requires histocompatibility laboratories to focus on epitope specificity determinations for serum antibodies. Highly reactive sera with complex reactivity patterns may need further testing, such as absorption-elution analysis with selected alleles, to separate antibody populations, and assessments should be made which antibody detection techniques provide the most clinically relevant information. Also, new standards and proficiency testing criteria need to be developed for epitope-based HLA antibody analysis.

With an epitope repertoire template for all potential donor HLA alleles, it seems quite easy to develop computer software that could identify from epitope specificity information, any allele as acceptable or unacceptable among a racially and ethnically diverse donor population. Not only can a suitable donor be more readily identified but one can also expect a better transplant outcome for the sensitized patient.

Desensitization of highly sensitized patients has been an alternative approach toward transplantation from a prospective donor. Many procedures using exchange plasmapheresis, intravenous immunoglobulin, bortezomib, and/or rituximab have shown to decrease donor-specific antibodies to levels considered acceptable for proceeding to transplantation. But often enough, donor-specific antibodies persist even after repeated efforts. Another way to assess the effectiveness of desensitization treatments could focus on the levels of antibodies specific for donor epitopes. Certain epitope-specific antibodies might disappear thereby opening new windows of opportunities to identify suitable donors. This "Match and Treat" strategy resembles the IVIG/plasmapheresis treatment of 2 highly sensitized pediatric patients who received a successful kidney transplant from donors selected with HLAMatchmaker.[77,84]

HLAMatchmaker is also being used to select donors for highly sensitized thrombocytopenic patients who are refractory to random donor platelet transfusions.[85-87] This refractoriness is often caused by HLA class I antibodies, and an epitope specificity analysis can identify compatible donors.[88]

A National Marrow Donor Program study of 2400 unrelated hematopoietic cell transplantation cases showed that class I triplet matching had only a modest effect on engraftment and reduced graft versus host disease and it did not improve patient survival.[89] This finding is not surprising because stem cell transplants are primarily affected by cellular immune mechanisms whereas HLAMatchmaker addresses only antibody-defined epitopes. Moreover, triplet matching does not predict in vitro alloreactive cytotoxic T cell responses.[90]

Epitope Immunogenicity and Control of HLA Sensitization

Many highly sensitized patients are retransplant candidates and although acceptable mismatching strategies and desensitization protocols may lead to another transplant, many patients do not benefit from these labor-intensive efforts. The enigma of the highly sensitized retransplant candidate must be considered an unacceptable problem. Why not make the first transplant more successful?

Obviously, transplanting from HLA matched donors offers a solution, but this can be done for a very small proportion of recipients because the HLA system is extremely polymorphic. Although new immunosuppressive treatments have improved long-term graft outcomes, they have side effects, such as toxicity and increased risks for infection and malignancy in transplant recipients.

As discussed above, the application of the eplet load concept to select HLA mismatches reflects a promising approach but it is probably not enough to achieve high levels of long-term transplant success. Tolerance induction strategies are being considered for kidney transplant patients who receive infusions of donor bone marrow-derived cells in combination with immunosuppression withdrawal with the goal of increasing allograft longevity. However, the frequent emergence of donor-specific antibodies prevents donor chimerism.[91] The successful induction of tolerance might be more readily achieved for donor mismatches with low eplet loads because they could reflect lower immunological barriers that might be easier overcome.[92] An analysis of the mismatched eplet repertoire of the transplant donor may also permit a determination which eplets have induced specific tolerance and which eplets have induced specific antibodies. Such information might also identify highly immunogenic eplets that adversely affect the success of a tolerance induction protocol.

Permissible HLA mismatching must focus on minimizing exposure to highly immunogenic eplets. Empirical studies determining the frequencies of antibody responses to mismatched eplets will provide information[23,26,93] but it is more important to reach a basic understanding of eplet immunogenicity.

As described in immunology textbooks, the antibody response begins with a B cell with immunoglobulin-like surface receptors that recognize specifically an epitope on the immunizing antigen. Such B cell is activated after complex formation between BCR and epitope. The interaction with helper T cells promotes the proliferation and differentiation (including affinity maturation) of the B cell and the subsequent plasma cell to produce antibodies.

The immunogenetic relationship between the HLA type of antibody producer and the immunizing allele determines conditions for antibody responses to HLA mismatches. Three complimenting theories address the HLA antibody response (Figure 1).

Our experience has shown that antibody-verified epitopes defined by pairs have a nonself eplet together with a self-configuration,[17,18] and this suggest that HLA antibody specificity has a self-reactivity component. This conclusion reflects the extensively discussed concept of nonself-self discrimination in immune responsiveness.[94-99] We have proposed the so-called nonself-self paradigm of HLA eplet immunogenicity which considers the hypothesis that the immune repertoire has B cells with low-avidity Ig receptors for self HLA epitopes.[12,14,100] Such receptors interact through their 6 Complementarity Determining Regions with a structural epitope comprising a configuration of multiple amino acid residues on the self HLA molecule but the binding strength is too weak for B cell activation and antibody production cannot occur. In contrast, exposure to a mismatched eplet can often induce a strong alloantibody response. The nonself-self paradigm explains that B-cell activation by a nonself eplet can only occur if the immunizing allele has a structural epitope configuration which consists primarily of self-residues shared with the antibody producer. This concept is consistent with recent data about epitope specificities that can only be explained with the nonself-self paradigm of HLA eplet immunogenicity.[12,101-105]

Does the nonself-self paradigm contradict the evidence that higher eplet loads lead to more antibody development? Not necessarily. The eplet load concept represents a risk assessment of an epitope-specific antibody response to a mismatched allele. All sera from sensitized patients, including those with high PRA values, have antibody specificities against 1 or very few epitopes.[80,81] A greater eplet load will more likely lead to an antibody response but it does not mean that lots of epitopes will be recognized. Specific generation of epitope-reactive antibodies will depend on the criteria of the nonself-self paradigm but this concept has yet to be validated experimentally.

A second theory proposed by Vasilis Kosmoliaptsis at the University of Cambridge addresses the concept that the relative antigenicity of an eplet can be predicted from atomic modeling and physiochemical properties of its amino acid residues.[25,29,106] Accordingly, quantitations of 3-dimensional surface electrostatic potentials of HLA eplets with the Poisson-Boltzmann equation[107,108] can explain the differential binding of epitopes with specific antibodies. Other physical properties of eplets, such as hydrophobicity, will also influence the binding with antibody. The physiochemical differences between nonself-eplet and self-eplets may provide the trigger for B-cell activation.[109]

Activated B cells need T cell help for their proliferation and differentiation into antibody-producing plasma cells. A third theory proposed by Eric Spierings at the University of Utrecht is referred to as the Predicted Indirectly ReCognizable Epitopes (PIRCHE) concept which addresses indirect T cell allorecognition of peptides generated by activated B cells after their uptake of the immunizing HLA antigen carrying the epitope specifically recognized.[110] Computer programs have been used to predict the numbers of immunizing antigen-derived peptides with relevant binding values with DRB1-alleles of the antibody producer. Only unique immunizer-specific peptide-DRB complexes are included in PIRCHE numbers. Donor class I allele antibody responses determined in sera after allograft nephrectomy correlated with larger PIRCHE numbers than the mismatched donor alleles which did not induce antibodies.[111] The PIRCHE effect was also shown for HLA antibodies after pregnancy.[112]

These theoretical concepts offer new perspectives of the very early phases of the HLA antibody response.[113] At present, we can only use indirect approaches such as serum antibody specificity analysis, molecular assessments of matching and structural analysis of HLA-antigen-antibody complexes to study the antigenicity and immunogenicity of HLA epitopes.

CONCLUSIONS

With the extensive documentation in this review, it has become apparent that epitope-based matching is superior to antigen matching. Are we ready to apply this principle in the clinical transplant setting?

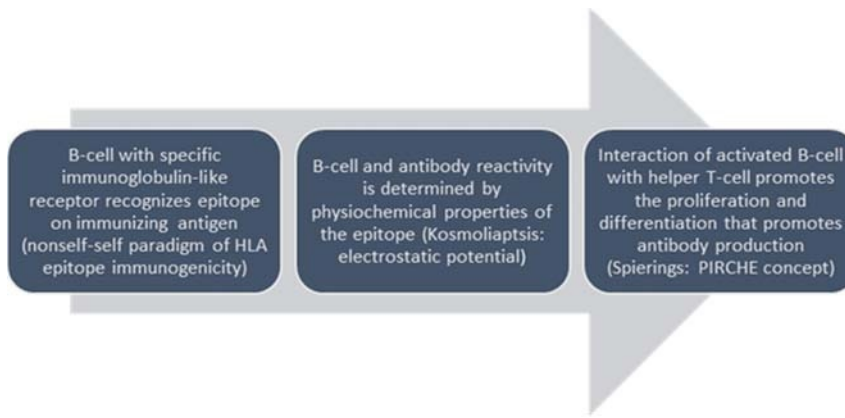

FIGURE 1. Three theories about eplet immunogenicity and how they relate to the dynamics of the antibody response to an HLA mismatch.

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