

## REVIEW

# The long and winding road towards epitope matching in clinical transplantation

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## SUMMARY

Recent data suggest that HLA epitope matching is beneficial for the prevention of *de novo* donor specific antibody (DSA) formation after transplantation. In this review, different approaches to predict the immunogenicity of an HLA mismatch will be discussed. The parameters used in these models are often called epitopes but the actual antibody epitope is far more complex. Exact knowledge of the antibody epitope is crucial if epitope matching is also used as a tool to select compatible donors for (highly) sensitized patients. Evidence is provided that it is not always possible to give an exact definition of an antibody epitope. We conclude that HLA “epitope” matching is superior over HLA antigen matching with respect to the prevention of *de novo* DSA formation and will enhance the prediction of acceptable HLA mismatches for sensitized patients. However, epitope matching at our current level of knowledge will not solve all histocompatibility problems as unexpected antibody reactivity still may occur.

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## Introduction

After the discovery that blood transfusion and pregnancy can lead to the induction of leucocyte-reactive antibodies [1–3], it soon became clear that the antigens recognized by these antibodies played a pivotal role in the immune response leading to graft rejection after kidney transplantation. When donors and recipients had the same HLA type, graft and patient survival was significantly better than in case of HLA mismatched transplants [4]. These first results were obtained with transplants using living related donors, often siblings of the patient, which made the chance of transplanting an HLA identical graft relatively high. Selection of an HLA identical unrelated donor is far more difficult, due to

the enormous polymorphism of the HLA system. Only in case a large pool of unrelated donors is available, full HLA matching on the serological level might become feasible. In order to reach this goal, the international organ exchange organization “Eurotransplant” was founded in 1967 by Jon van Rood [5]. By creating a common waiting list and a common donor pool of several countries in Europe, HLA matching became a realistic option, at least for a subpopulation of patients. It appeared that this initiative was successful, as about 20% of the patients within Eurotransplant were transplanted with an HLA-A, -B, -DR identical donor, which was associated with superior graft survival compared to HLA mismatched transplants [6]. Unfortunately, still the majority of patients were transplanted with a

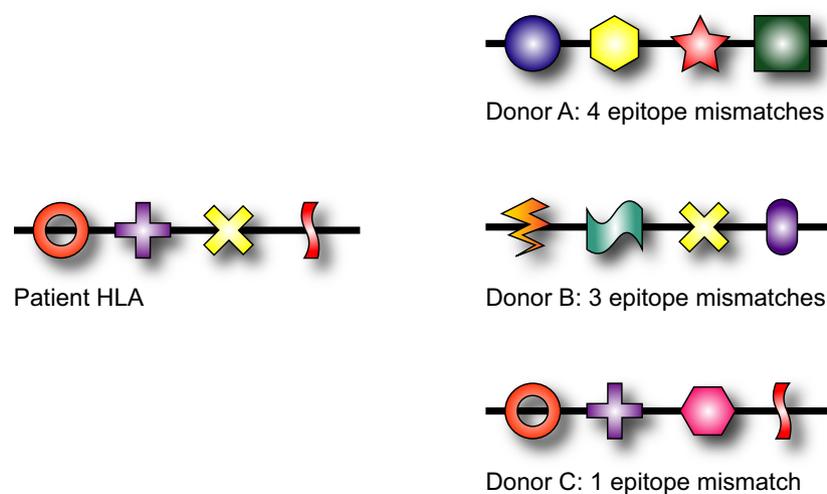
(partially) HLA mismatched graft. Nonetheless, it appeared that decreasing the number of HLA mismatches was already a tool to minimize sensitization and prevent early graft loss. During the years following, more efficient immunosuppression became available leading to prolonged graft survival, also in HLA mismatched transplants. Nevertheless, sensitization towards HLA still occurs, and those patients are at risk for early graft loss [7]. Moreover, once sensitized the chance of finding a suitable donor organ for a patient becomes far more difficult. Recently, several approaches of alternative HLA matching have been described to prevent the induction of donor specific antibody (DSA) in order to improve graft survival. Knowledge of the exact amino acid sequence of the different HLA antigens appears to be crucial for the selection of an optimally HLA mismatched donor [8]. In this review, we will focus on the differential immunogenicity of epitopes, the requirement for T cell help and the difficulties in determining the exact binding determinants of HLA antibodies.

### From HLA antigens towards HLA epitopes

Soon after the serological identification of HLA antigens it became clear that HLA molecules belonged to a highly polymorphic system. While the introduction of molecular typing was crucial for a more accurate definition of HLA antigens, it also resulted in an enormous increase of the number of HLA alleles reported. At the moment, more than 15 000 alleles are known and it is to be expected that this number will increase

far more by broader application of next generation sequencing as a tool for HLA typing. At first glance, these developments make the selection of an HLA mismatched donor organ that will not induce DSA a mission impossible.

Fortunately, knowledge on the exact amino acid sequence of the different HLA alleles has given more insight in the crucial positions on the HLA molecules for the induction and reactivity of allo-antibodies. Already in the early days of HLA, when the HLA antigens were still serologically characterized with allo-antisera, it became clear that the different HLA molecules share antigenic determinants that we now call epitopes [9]. Several CREGs (Cross REactive Groups) could be identified based on their shared reactivity with the same allo-antibodies [10]. However, for an optimal characterization of the epitopes expressed by the different HLA antigens, molecularly HLA typing at the allele level is crucial [8]. Nowadays it is clear that every HLA antigen consists of a unique set of antibody epitopes, while the individual epitopes can be shared by multiple HLA antigens [11]. The consequence is that the number of foreign antibody epitopes varies within the same level of HLA antigen mismatches (Fig. 1). Some HLA mismatches have many epitopes not shared by the HLA antigens of the patient. These mismatches are likely to be more immunogenic compared to an HLA mismatch which shares most epitopes with the patient. Several tools have been developed to determine the relative immunogenicity of an HLA mismatch on basis of this principle.



**Figure 1** HLA alleles can be considered as a string of potential antibody epitopes. A specific HLA allele consists of a unique set of epitopes while the individual epitopes can be shared with other alleles. The consequence is that the number of foreign epitopes on an individual HLA mismatch can differ and depends on the HLA type of the potential antibody producer. It is to be expected that the HLA mismatch of donor A (four foreign epitopes for the patient) is more immunogenic than the one of donor C (only one nonself epitope).

## Tools to define the immunogenicity of an HLA mismatch

The first individual who realized that the patchwork character of HLA molecules with respect to antibody epitopes could provide a basis to determine the immunogenicity of foreign HLA molecules, was Rene Duquesnoy in Pittsburgh. He defined HLA molecules as strings of crucial amino acid polymorphisms, which potentially can trigger an antibody response [12]. Originally these polymorphisms were defined as consisting of three amino acids, adjacent to each other on antibody accessible sites of the molecule, called triplets. Later, Duquesnoy redefined the crucial polymorphism as amino acids within 3 Ångstrom radius, structurally near to each other on the tertiary conformation of the HLA molecule, called eplets [13]. The computer algorithm HLAMatchmaker made it possible to compare the triplets or eplets on a foreign HLA molecule with those present on the patient's own HLA molecules [14]. During the past years, several studies demonstrated clear associations between the number of triplets or eplets on a mismatched HLA antigen and the chance that a patient develops *de novo* DSA [15–18]. Both in case of mismatched HLA class I and HLA class II antigens, the incidence of antibody formation increases with the number of foreign triplets or eplets. Some studies even suggest that it is possible to define a threshold of a certain number of eplets, which predicts whether antibody production will occur [19]. However, even a very low number of mismatched eplets can already give rise to DSA formation as was already shown by the studies of Dankers *et al.* [16], which demonstrated a clear association between the number of mismatched triplets and the chance that a patient will develop *de novo* DSA. In case of 12 or more triplet mismatches 100% of the patients, who rejected their graft, had developed DSA but also in case of one or two triplet mismatches respectively 10% and 22% of the patients produced DSA. Furthermore, it remains to be established whether the definition of the number of foreign eplets is the optimal way to predict the immunogenicity of an HLA antigen. A similar predictive value has been demonstrated if one considers the total number of antibody-accessible amino acid substitutions of the mismatched HLA antigen in comparison with the own HLA antigens of the patient [20].

A completely different approach has been developed by the Cambridge group. Their studies show a clear role of the physiochemical properties of mismatched amino acids. If the physiochemical properties of mismatched

amino acids are very different from those of the own HLA antigens of the patients, induction of donor specific antibodies is far more likely than in case of similar properties [20–22]. They have validated a score system (EMS-2D), which determines the degree of foreignness of the physiochemical characteristics, both for the induction of DSA after renal transplantation and after blood transfusion. The higher the score, the more likely it is that a patient will make antibodies.

The different approaches, which consider polymorphisms on antibody accessible sites of the donor HLA molecule, all suggest that quantitative aspects i.e. the number of foreign triplets, eplets, amino acids play a crucial role but do not consider a potential difference in the immunogenic properties of the individual polymorphisms [23].

## Differential immunogenicity of individual "epitopes"

Although the relationship between the number of triplet/eplet mismatches and the chance to develop DSA is well established, the immunogenicity of the individual triplets/eplets may vary. Not every polymorphic site has the same immunogenic potential and one could hypothesize that a higher number of triplet/eplet mismatches makes it likely that one of these is particularly immunogenic. This is also suggested by a study on the development of DSA associated with graft loss in a cohort of 1311 previously nonimmunized males, who returned on the waiting list of Eurotransplant after failure of their first transplant. This study, which focused on the development of antibodies against HLA-A and -B showed that some mismatched triplets led to antibody formation in about 50% of the cases whereas others led to a lower incidence of DSA or hardly induced any antibodies (Table 1). Similarly to the frequencies of HLA alleles, which differ amongst populations, the frequency of potential antibody epitopes will also be different between populations in the world ([www.allelefrequency.net](http://www.allelefrequency.net)). The chance that a particular HLA allele mismatch will induce antibodies depends amongst others on the frequency of its most immunogenic epitope in the population. If this frequency is high, it is more likely that donor and recipient share this epitope, resulting in a low number of patients with that antibody specificity. If the frequency of the immunogenic epitope is low in a population, the incidence of antibody formation against that HLA allele in case that it is mismatched will be high. A preliminary study comparing the incidence of antibody formation in transplant recipients in Israel

**Table 1.** The immunogenicity of individual epitopes differs as demonstrated by analyzing the epitope specificity of donor specific antibody (DSA) developed in previously nonsensitized males, who returned on the waiting list after failure of their first kidney transplant

Positions + amino acids	Yes DSA	No DSA	% DSA	Donor mismatches
79G 80T 81L	30	23	56.6	A23 A24 A25 A32
11A 12M 13S	55	49	52.9	B7 B18 B27 B37 B38 B39 B46 B48 B61
150A 151H 152V	104	95	52.3	A1 A3 A11 A23 A25 A26 A29 A30 A31 A32 A33
126L 127K 128E	102	94	52	A1 A3 A11 A25 A26 A29 A30 A31 A32 A33
130L 131S 132S	30	30	50	B7 B8
73I 74D 75R	14	47	23	A31 A3375
73R 76E 77D	20	69	22.5	B27, B37, B47
185P 186R 187T	1	15	6.3	A33

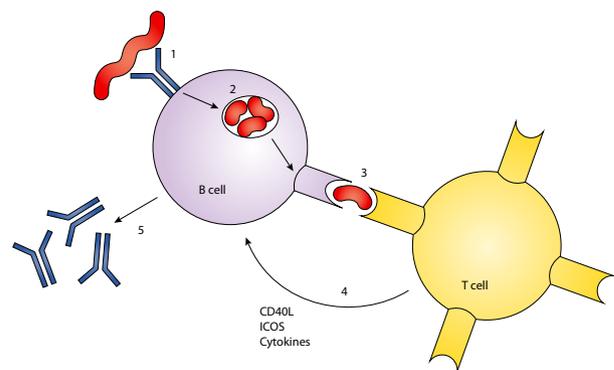
Very immunogenic epitopes are associated with specific antibodies in more than 50% of the cases at risk whereas other epitopes are less immunogenic.

versus Eurotransplant confirms this hypothesis (Israeli *et al.* manuscript in preparation). These data suggest that future matching strategies should not only focus on the number of mismatched eplets, triplets or amino acids but, especially, on prevention of mismatches for highly immunogenic polymorphisms. In order to be able to reach this goal, it is crucial to identify the immunogenicity of the different polymorphisms with respect to the induction of allo-antibodies. This is one of the aims of the 18th International Histocompatibility workshop, which will take place in Amsterdam in 2021. By collecting information on the incidence of *de novo* DSA in a large group of high resolution typed donor-recipient combinations from different populations, it should be feasible to identify the most immunogenic antibody epitopes. In future allocation, these should be avoided as a mismatch in order to prevent the induction of DSA. The other side of the coin is the identification of polymorphisms, who do not lead to allo-antibodies in an individual patient and are likely to serve as acceptable mismatches.

### An additional role for T cell epitopes

The majority of the donor specific antibodies that develop after transplantation belong to the IgG class. Production of IgG antibodies is only feasible if CD4<sup>+</sup> T cells provide help to the B cells, as without such an interaction activation of B cells will only lead to IgM antibodies. T cell help is based on the recognition of peptides derived from allogeneic HLA molecules presented by the HLA class II molecules on the B cell (Fig. 2). Whereas, B cell epitopes solely reside on antibody accessible locations, polymorphisms throughout

the HLA molecule can theoretically give rise to T cell epitopes. Indeed, studies on the location of B cell and T cell epitopes showed overlapping regions, as one would expect, but also the presence of T cell epitopes in areas where no B cell epitopes were present [24]. Identification of the actual peptides, which function as targets for this so called indirect allorecognition by CD4<sup>+</sup> T cells, has shown to be very difficult due to the low frequency of indirectly recognizing T cells [25]. Nonetheless, a computer algorithm has been developed, which calculates the number of potential allogeneic peptides derived from the mismatched HLA molecule that are able to bind to the HLA-DR molecules present on the B cells of



**Figure 2** The production of IgG antibodies depends on a specific interaction between CD4<sup>+</sup> T cells and B cells. 1: The B cell receptor recognizes an epitope on a foreign HLA molecule. 2: This leads to internalization of the target antigen, which is then degraded into peptides. 3: Some of these peptides bind to the HLA class II molecules on the B cell and the foreign (nonself) peptides are recognized by CD4<sup>+</sup> T cells. 4: This leads to activation of the T cells associated with the production of immunoregulatory molecules. 5: These molecules trigger a class switch of the antibodies produced.

the recipient [26]. This PIRCHE approach (**P**redictable **I**ndirectly **R**eCognizable **H**LA **E**pitopes) has shown to be of additional value for the prediction of the chance that donor specific antibodies will be produced [26–28]. An increased number of theoretical T cell epitopes is associated with a higher chance that a patient will start to make antibodies to a mismatched HLA antigen. A recent publication showed that the number of PIRCHE mismatches and the number of eplet mismatches were independent risk factors for both the development of DSA after transplantation, and graft outcome [18]. Similar to B cell epitopes, the challenge is to discriminate the actual immunogenic PIRCHEs from the nonimmunogenic theoretical ones. It is to be expected that a combinatorial approach, focussing on the identification of both B cell and T cell epitopes, will be the optimal basis of future matching strategies.

### Immunogenic determinants versus actual antibody epitopes

So far, the immunogenicity of an HLA mismatch for the humoral immune response has been discussed with a special emphasis on the predictive value of polymorphic structures which serve as a trigger for, or are associated with, the induction of an antibody response. As discussed above, these structures are rather simple and consist of one or a few amino acids. However, the actual antibody epitope, which consists of the crucial contact sites on the HLA molecule necessary for the interaction with antibody molecule is far more complex. In principle, an antibody molecule has a paratope, consisting of six so called complementary determining regions (CDRs), three on the heavy chain and three on the light chain, which interact with the HLA antigen recognized. The interaction of the epitope with these CDRs leads to a kind of footprint on the HLA molecule (Fig. 3). The specificity of the antibody is determined by the amino acids on the HLA molecule, which interact with the CDR3 on the heavy chain of the immunoglobulin molecule. These amino acids include the triplets/eplets, which were responsible for the induction of the antibody response. The other contact sites contribute to the stability and avidity of the interaction between antibody and antigen. In order to be able to predict the HLA antigen reactivity pattern of an antibody, it is essential to determine which of these interactions are crucial for proper binding of the antibody. In some cases, antigens only sharing the polymorphism interacting with the CDR3 with the immunizing antigen, are already targets for an alloantibody. In other

cases an additional requirement is that one or more other CDRs bind exactly the same amino acids as present on the immunizing antigen. The use of human HLA-specific monoclonal antibodies (mAbs) has been instrumental for the definition of actual antibody epitopes, while also absorption elution experiments are suitable to define the crucial polymorphisms defining an antibody epitope. A dedicated website has been developed to register the actual antibody epitopes on the different HLA antigens [29,30].

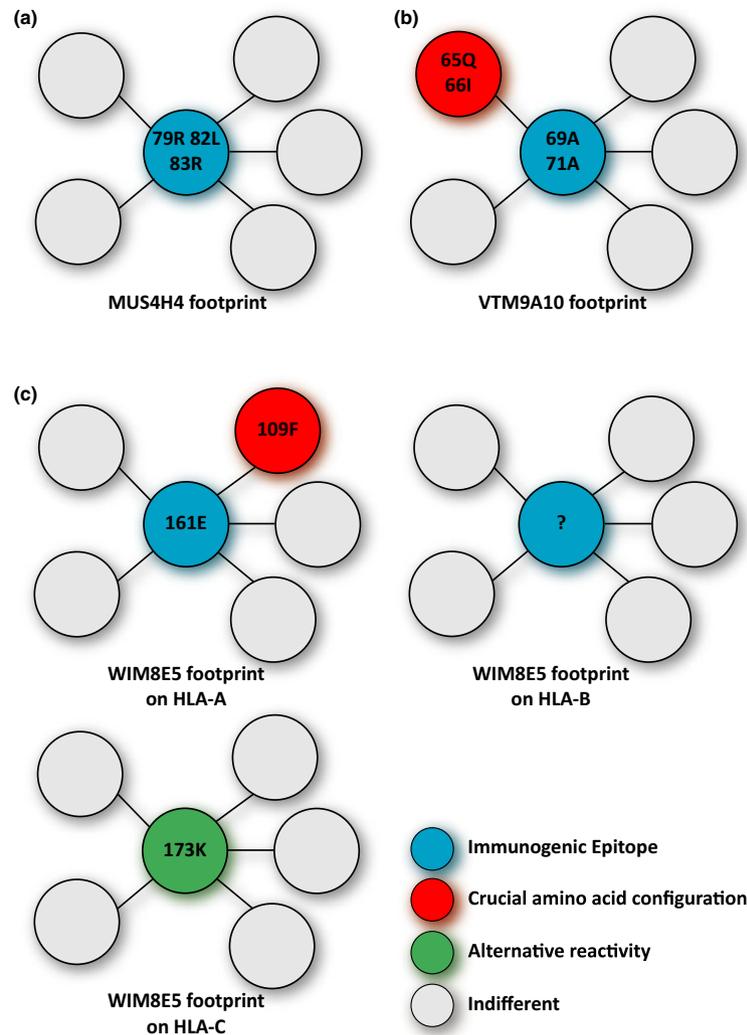
It is clear that some of the epitopes can be easily defined, while others are more complex, as shown below.

### Definition of the actual HLA antibody epitope can be difficult, even with monoclonal antibodies

Both human mAbs and absorption/elution studies with alloantisera have been very instrumental for the definition of the actual antibody epitopes on the HLA molecules [31–33]. These verified epitopes are registered at the epitope registry (<http://www.epregistry.com.br>) As mentioned above, antigen-antibody interaction involves six CDRs of which the CDR3 binds to the immunogenic epitope and determines the specificity of the antibody. In most cases, the presence of this immunogenic epitope, alone or in combination with one or two other polymorphic sites, can explain the antibody reactivity pattern with the different HLA alleles (Table 2, Fig. 3a, b, monoclonal antibodies MUS4H4 and VTM9A10). However, sometimes very complicated reaction patterns are observed, which make it virtually impossible to define an epitope that explains all antibody reactivities.

Here we describe an example of an HLA-A\*11:01 induced mAb, WIM8E5, derived from a woman who became sensitized during pregnancy by paternal antigens. The trigger of the antibody response can be any epitope on the mismatched HLA, dependent on the phenotype of the mother. In this case, comparison of all the HLA class I molecules of the mother with the immunising HLA-A\*11:01, reveals that there are only two amino acid differences, which may have triggered the antibody response. However, if we compare only the HLA-A molecules of the mother with HLA-A\*11:01, there are six amino acid differences.

When WIM8E5 was screened with single antigen bead (SAB) assays of two independent vendors, a broad HLA antigen reactivity was observed, including specificities of all three classical HLA class I loci. In contrast to other mAbs, the reactivity of WIM8E5



**Figure 3** Foot prints of three human monoclonal antibodies on their target antigens. (a) The reactivity of monoclonal MUS4H4 depends only on sharing of the immunogenic epitope with the HLA molecule, which has triggered the production of this antibody. (b) For the reactivity of monoclonal antibody VTM9A10 sharing of both the immunogenic epitope and an additional contact site with the immunizing antigen is crucial. (c) The reactivity of monoclonal antibody WIM8E5 is very complex. It appears that the reactivity with HLA-A antigens depends on sharing of the immunogenic epitope and an additional contact site. The observed cross-reactivity with HLA-B and -C antigens has completely different requirements. The reactive HLA-C alleles have one particular polymorphic position in common (in green) whereas the basis of the reactivity with HLA-B targets remains unclear. Note: more details on the immunizing effect leading to the production of these antibodies is given in table 2.

**Table 2.** HLA types of the antibody producer and immunizer and epitope specificity of the three monoclonal antibodies depicted in Fig. 3

Human mAb	HLA antibody producer	HLA immunizer	Epitopes on reactive HLA class I allele		
			HLA-A	HLA-B	HLA-C
MUS4H4 (IgG)	A*02:01, A*26:01, B*39:01, B*41:01, C*12:03, C*17:01	A*24:02	79R82L83R	79R82L83R	–
VTM9A10 (IgG)	A*25:01, A*29:02, B*44:03, B*15:01, C*05:01, C*16:01	B*07:02	–	69A71A (65Q66I)	–
WIM8E5 (IgG)	A*03:01, A*03:02, B*47:01, B*51:01, C*06:02, C*15:02	A*11:01	161E(109F)	?	173K

? indicates the epitope recognized on HLA-B is unclear.

cannot be explained by the fact that the reactive alleles share the immunogenic epitope and some other crucial amino acid configurations, which are absent on the nonreactive antigens. Almost all HLA-A antigens are reactive, except for three, including the self-antigens. In addition, some HLA-B and HLA-C antigens were reactive. Extensive comparisons of the amino acid composition of the different reactive HLA antigens did not lead to a clear definition of an antibody epitope (Fig. 3c).

Others have shown that dilution of serum samples can clarify the patterns observed in SAB assays [34]. Dilution of WIM8E5 led to a decrease in the breadth of reactive HLA antigens. One group of HLA-A antigens, including the immunising HLA-A\*11:01, remained highly reactive in SAB assays upon dilution. The reactivity of the other HLA-A antigens was less consistent and decreased with each dilution step, while the reactivity of the HLA-B and HLA-C antigens decreased rapidly upon dilution.

Most of the HLA class I epitopes have been defined on the basis of interlocus comparisons of amino-acid sequences. However, for WIM8E5 the reactive HLA-C antigens do not have any amino acid configuration in common with the reactive HLA-A antigens. However, they do share a unique amino acid that is absent on the nonreactive HLA-C antigens. These data suggest that the induction of the WIM8E5 antibody was induced by an epitope mismatch specific for HLA-A and that the cross-reactivity with HLA-B and HLA-C is not based on reactivity of the antibody with the same, HLA-A specific epitope.

This example shows that verification of HLA epitopes using mAbs involves more than screening the mAb with SAB assay, and just identifying the shared amino acid configurations of the reactive antigens. The different reactivity patterns upon dilution of WIM8E5 do support the conclusion that different epitopes are involved and that the affinity of the antibody for the different target antigens is different. An alternative explanation for the observed cross-reactivity might be that the reactive antigens have similar physicochemical properties, despite amino acid variation within the critical contact site.

### Concluding remarks

For many years, HLA matching strategies have focused on the selection of donors with a minimal number of HLA antigen mismatches. However, it is clear that the immunogenicity of mismatched HLA antigens can differ. If one would like to prevent the induction of *de*

*nov* DSA, which is known to be associated with a poor outcome, then limiting the number of HLA antigen mismatches is not the optimal strategy. Our view on HLA antigens as possible targets for antibodies has been changed considerably over time. Antibodies are not specific for an HLA antigen but for an epitope present on the HLA molecule. Every HLA antigen can be considered as a string of antibody epitopes. A particular HLA antigen consists of a unique combination of epitopes while the individual epitopes can also be expressed on other HLA antigens. This is the reason why the number of foreign epitopes present on a mismatched HLA antigen will differ and depends on the HLA type of the recipient. Some HLA mismatches express many epitopes whereas others have only a few or even no foreign epitope. As a consequence, the presence of two HLA antigen mismatches on a donor organ may, for some recipients, be associated with fewer epitope mismatches than the presence of a single antigen mismatch. Therefore, novel matching strategies aim at the limitation of the number of foreign epitopes rather than the number of HLA antigen mismatches.

Current activities in the field of epitope matching can be divided in two categories. The first one is aiming at the prevention of *de novo* DSA formation by limiting the number of potentially antibody inducing polymorphisms on the mismatched HLA molecules. Indeed, several tools have been described, which can successfully predict the chance that DSA will be induced [23]. These include the number of foreign eplets/triplet, amino-acids or PIRCHE's, and the physicochemical properties of the mismatched HLA molecule. Such parameters are already excellent predictors of the immunogenicity of an HLA mismatch and as soon as we are able to distinguish the very immunogenic polymorphisms from the nonimmunogenic ones, their prediction will be even better. However, none of these parameters can be considered as the actual antibody epitope. Exact knowledge of the antibody epitope is crucial if one would like to introduce epitope matching as a tool for virtual cross-matching of (highly) sensitized patients. Human monoclonal antibodies and absorption/elution studies have been very helpful for the definition of several antibody epitopes, which are described in the HLA epitope registry. At the moment, this collection is far from complete and the example described in this review suggests that it will not always be feasible to explain the possible reactivity of an HLA antibody based on the knowledge of the amino acid sequence of the immunizing antigen.

In conclusion, “epitope” matching is superior over antigen matching with respect to the prevention of *de novo* DSA formation and will enhance the prediction of acceptable HLA mismatches for sensitized patients. However, one should realize that epitope matching at our current level of understanding will not solve all histocompatibility problems, as unexpected antibody reactivity still may occur.

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