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REVIEW ARTICLE

Antibody-reactive epitope determination with HLAMatchmaker and its clinical applications

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Abstract

Antibodies against allogeneic human leukocyte antigen (HLA) molecules are important impediments to the success of different clinical procedures including transplantation and platelet transfusion. In these settings, characterization of the repertoire of immunogenic epitopes is important for permissible mismatch determination and the identification of acceptable mismatches for sensitized patients. HLAMatchmaker is a computer algorithm that considers small configurations of polymorphic residues referred to as eplets as essential components of HLA epitopes. This review critically elaborates the concepts underlying the HLAMatchmaker and describes the usefulness of HLAMatchmaker in the clinical setting. Recent developments have increased our understanding of structural basis of HLA antigenicity (i.e. reactivity with specific antibody) and immunogenicity (i.e. its ability to induce an antibody response).

Introduction

Human leukocyte antigen (HLA) antibodies represent significant risk factors for transplant failure and refractoriness to platelet transfusions. Such antibodies are the result of immune responses to mismatched HLA antigens which can occur after transplantation as well as before transplantation following blood transfusions or during pregnancy.

The traditional description of antibody specificity is based on the reactivity with HLA antigens such as anti-A1, anti-B7, and anti-DR1 and antibody reactivity patterns with multiple HLA antigens categorized into serologically cross-reacting groups such as A2-CREG and B7-CREG. Accordingly, HLA antibodies could be specific for private determinants unique to individual antigens or to public determinants shared by cross-reacting antigens. This experience made us aware that HLA antigens carry multiple serologic epitopes but their precise characterization remained elusive until determinations of the stereochemical structure of crystallized HLA molecules and amino acid sequence differences between HLA antigens. Since the late 1980s, many studies with alloantibodies and mouse monoclonal antibodies have shown that private and public epitopes correspond to distinct amino acid polymorphisms on the HLA molecular surface. With the realization that HLA antibodies recognize epitopes rather than antigens or CREGs, it has become necessary to develop new criteria to interpret the serum reactivity of sensitized patients. HLAMatchmaker represents an epitope-based approach to assess HLA mismatch acceptability and select suitable donors for alloimmunized patients in need of an organ transplant or requiring matched platelet transfusions (1, 2).

Two recent Current Opinion reviews describe the concept of HLAMatchmaker and its usefulness in HLA epitope matching for organ transplantation (3, 4).

Brief description of HLAMatchmaker

HLAMatchmaker is a theoretical algorithm whereby each HLA antigen is viewed as a string of amino acid configurations in antibody-accessible positions; they are considered key elements of epitopes that can elicit specific alloantibodies. The original version used triplets, i.e. linear sequences of three residues (1), but provided an incomplete description of the HLA epitope repertoire. The so-called eplet version is based on the stereochemical modeling of protein antigen–antibody complexes and the contributions of critical amino acid residues that dominate in antigen–antibody binding (2). The residues of such patches are within a 3-Å

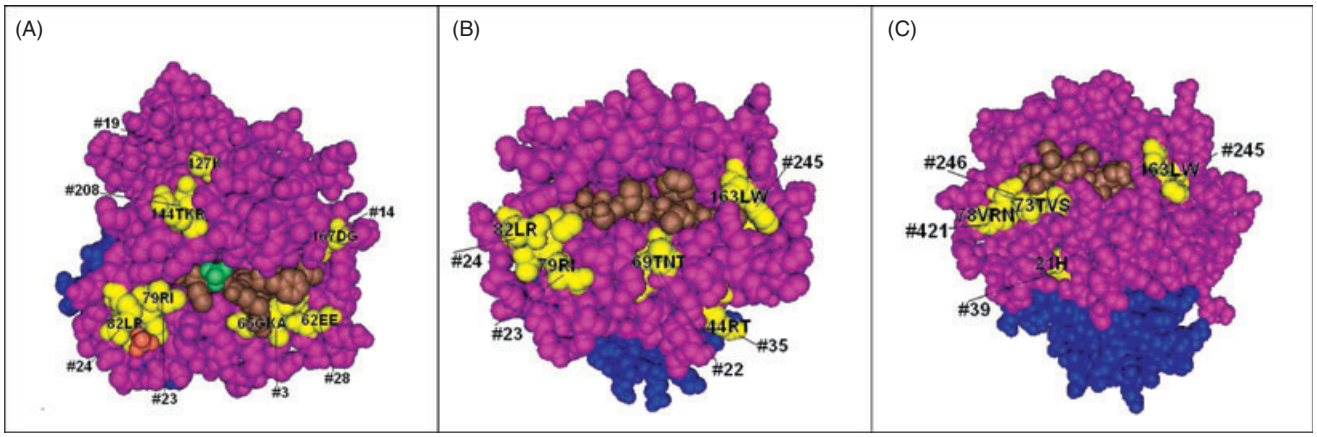

Figure 1 Examples of molecular locations of class I eplets that correspond to antibody-verified epitopes (Table 3). (A) HLA-A24, (B) HLA-B51, and (C) HLA-Cw3. These eplets correspond to Terasaki's epitope numbers described by El-Awar et al. (44).

radius and at least one of them is non-self. Figure 1 shows the locations of selected class I eplets that correspond to epitopes verified experimentally by specific antibodies. Each eplet is assigned a position number in the amino acid sequence and the notation system lists only polymorphic residues marked with the standard letter code. Many eplets are identical to triplets but others have residues in discontinuous sequence positions that cluster together on the molecular surface. Serologically defined HLA determinants correspond well to eplets. The eplet version of HLAMatchmaker therefore represents a more complete repertoire of structurally defined HLA epitopes and provides a more detailed assessment of HLA compatibility.

Class I HLA molecules have 75 polymorphic positions from which we determined 199 eplets on HLA-A, -B, -C, 110 are on the α-helices, 60 are on the side surface, and 29 are in less accessible positions at the bottom and under the peptide-binding groove (2). Class II antigens have 44 DRB, 33 DQB, 29 DQA, 20 DPB, and 9 DPA polymorphic positions that contribute a repertoire of 146 DRB, 74 DQB, 58 DQA, 45 DPB, and 19 DPA eplets (5). An analysis of 56 major histocompatibility complex class I -related chain A (MICA) alleles has identified 38 MICA eplets considered as potential epitopes that can induce specific alloantibodies (6).

HLAMatchmaker applies two principles: (i) each HLA represents a distinct string of structurally defined eplets as potential immunogens that can induce specific antibodies and (ii) patients cannot make antibodies against epitopes that are expressed by their own HLA molecules. The algorithm assesses donor–recipient compatibility through intralocus and interlocus comparisons and determines what eplets on mismatched HLA molecules are different or shared between donor and patient. An understanding of the HLA antibody response must consider the notion that a mismatched antigen has two characteristics, namely antigenicity (i.e. the reactivity with antibody) and immunogenicity (i.e. the ability of inducing an antibody response). Immunogenicity depends on the structural difference between an immunizing protein and the antibody responder's homologous proteins.

Mismatch immunogenicity and epitope load

HLAMatchmaker can be used as a quantitative tool to determine the degree of a mismatch (i.e. the number of mismatched eplets or triplets). A given HLA mismatch has an epitope load that is primarily determined by the recipient's HLA type representing a repertoire of self-epitopes to which no alloantibodies can be made. For some patients, a mismatched antigen might be structurally compatible whereas for other patients it has multiple mismatched epitopes (3). Table 1 shows examples of eplet mismatches of three class I alleles for eight HLA phenotypes. For each allele, one can readily identify cases with low or high eplet loads.

The incidence of the anti-class I antibody response induced by a transplant or during pregnancy correlates with the number of non-self-triplets or eplets on mismatched antigens (7–10). Anti-HLA-C antibody responses by patients with rejected kidney transplants also correlate with eplet loads of HLA-C mismatches (11).

Conventional class II matching criteria consider only HLA-DR antigen, but this approach is an insufficient reflection of histocompatibility because antibodies against other class II mismatches including DP and DQ have been shown to diminish transplant success. Each DR antigen should be viewed as a package of DR+DQ+DP antigens and the overall class II epitope load depends on the patient's DR, DQ, and DP types. Table 2 illustrates how certain DR haplotypes have considerable epitope loads especially if DRB3/4/5, DQB, and DQA are included. For a DR15,18 recipient (case 1), the haplotypes of the DR1 and DR8 mismatches have the fewest eplet mismatches, whereas the DR4, DR7, and DR9 haplotypes have the highest numbers of mismatched eplets. DR15 would be considered a match, but in this case, the donor's

Table 1 Examples of eplet loads of allele mismatches for different HLA phenotypes

Case	Recipient HLA phenotype	*A*32:01*		*B*56:01*		*C*08:02*	
		No.	Mismatched eplets	No.	Mismatched eplets	No.	Mismatched eplets
1	*A*02:01, *25:01;B*08:01, *35:01;C*04:01, *07:01*	1	62QE	4	65QIA,69AA,70AQA,71AT	6	69RT,71AT,76TVS, 138K,177KT,275GP
2	*A*02:01,*26:01;B*07:02,*45:01;C*05:01,*07:02*	4	62QE,77ESI,79RI, 82LR	1	163LW	1	76TVS
3	*A*01:01,*25:01;B*13:01,*37:01;C*07:04,*12:03*	0		9	44RE,63RNI,65QIA, 69AA,70AQA,71AT, 77ESN,161EL,163LW	7	69RT,71AT,73TV,76TVS, 138K,156RA,275GP
4	*A*02:01,*03:01;B*07:02,*45:01;C*03:03,*07:02*	5	69NAH,77ESI,79RI,82LR, 246S	0		3	138K,177KT,275GP
5	*A*33:01,*68:02;B*14:01,*45:01;C*03:03,*05:01*	6	9F,62QE,73ATS, 77ESI,79RI,82LR	4	65QIA,69AA,70AQA,73ATS	0	
6	*A*11:01,*30:01;B*15:01,*51:01;C*01:02,*02:02*	10	69NAH,70HS,73ATS,77ESI, 151ARV,184A,193AV, 207S,246S,253Q	6	44RE,65QIA,69AA, 70AQA,73ATS,151ARV	4	12AVR,138K,177KT,275GP
7	*A*69:01,*74:01;B*18:01,*27:05;C*14:02,*17:01*	3	73ATS,77ESI,79RI	5	11AM,70AQA,73ATS, 161EL,163LW	3	138K,177KT,275GP
8	*A*23:01,*80:01;B*42:01,*53:01;C*16:01,*18:01*	6	62QE,77ESI,184A, 193AV,246S,253Q	0		3	138K,177KT,275GP

HLA, human leukocyte antigen.

DR15 haplotype has six mismatched eplets because of the DQB difference with the recipient. For a DR11,16 recipient (case 2), DR12 and DR15 have the fewest eplet mismatches and DR7 and DR9 have the most. The donor's *DRB1*16:02* has two eplet differences with the recipient's *DRB1*16:01*. This *DRB1*16:02* haplotype also has a different *DQB1*03:01* and *DQA1*05:01*, but they do represent eplet mismatches because the recipient's *DRB1*11:01* haplotype has the same DQ alleles. These examples are merely intended to illustrate that high-resolution DR, DQ typing can provide detailed class II matching information at the eplet level.

Epitope loads of class II mismatches also affect specific antibody responses. Donor-specific, DRB1-reactive antibodies are less often detectable than antibodies against other class II epitopes (12). Antibody absence correlates with low numbers of mismatched DRB1 eplets. In contrast, donor-specific DRB3, 4, and 5 mismatches induce more antibody responses and they have higher numbers of incompatible eplets. Anti-DQ antibodies are rather common and this correlates with more mismatched eplets on DQB and DQA than on DRB1 (12). About one-third of class II sensitized patients have anti-DP antibodies reactive with a few DPB eplets and an allelic pair of DPA eplets.

HLA epitope load and clinical applications

Information about epitope loads of HLA mismatches is of potential clinical utility in any setting of cellular therapy involving the transfer of allogeneic tissues into a patient. The most widely studied of these settings are transplantation and platelet transfusions.

HLA epitope load and transplantation

In the management of transplant patients, epitope loads can be interpreted as risk factors for antibody-mediated rejection in the clinical management of transplant recipients and may eventually lead to new strategies for HLA mismatch permissibility to reduce alloimmunization and increase transplant survival.

Kidney transplants

A 2003 analysis of the United Network for Organ Sharing and Eurotransplant kidney transplant databases showed that HLA-A,-B mismatched kidneys with low triplet loads have the same graft survival rates as the zero HLA-A,-B antigen mismatches (13). This beneficial effect of triplet matching applies to both non-sensitized and sensitized patients and also to White and non-White patients. Thus, HLAMatchmaker can be used to increase the number of suitably matched kidney donors (14).

Haririan et al. (15) have also shown that triplet matching can provide useful prognostic information about kidney transplantation in African-Americans. Although a study of

Table 2 Examples of class II eplet load differences among serologically defined DR antigens

Donor DR	DRB1	DRB3/4/5	DQB1	DQA1	Eplet total	DRB1 eplets	DRB3/4/5 eplets	DQB1 eplets	DQA1 eplets
Case 1: DR15,18[a]									
DR1	*DRB1*01:01*	None	*DQB1*05:01*	*DQA1*01:01*	9	5	0	2	2
DR4	*DRB1*04:01*	*DRB4*01:01*	*DQB1*03:01*	*DQA1*03:02*	42	8	14	9	11
DR7	*DRB1*07:01*	*DRB4*01:01*	*DQB1*02:02*	*DQA1*02:01*	41	10	14	10	7
DR8	*DRB1*08:01*	None	*DQB1*04:02*	*DQA1*04:01*	4	4	0	0	0
DR9	*DRB1*09:01*	*DRB4*01:01*	*DQB1*03:03*	*DQA1*03:02*	36	6	14	5	11
DR10	*DRB1*10:01*	None	*DQB1*05:01*	*DQA1*01:01*	12	8	0	2	2
DR11	*DRB1*11:01*	*DRB3*02:02*	*DQB1*03:01*	*DQA1*05:01*	22	3	2	9	8
DR12	*DRB1*12:01*	*DRB3*02:02*	*DQB1*03:01*	*DQA1*05:01*	26	7	2	9	8
DR13	*DRB1*13:01*	*DRB3*01:01*	*DQB1*06:03*	*DQA1*01:03*	12	2	0	7	3
DR14	*DRB1*14:01*	*DRB3*02:02*	*DQB1*05:03*	*DQA1*01:04*	11	4	2	2	3
DR15	*DRB1*15:01*	*DRB5*01:01*	*DQB1*06:02*	*DQA1*01:02*	6	0	0	6	0
DR16	*DRB1*16:02*	*DRB3*02:02*	*DQB1*03:01*	*DQA1*05:01*	22	3	2	9	8
DR17	*DRB1*03:01*	*DRB3*01:01*	*DQB1*02:01*	*DQA1*05:01*	17	0	0	9	8
DR18	*DRB1*03:02*	*DRB3*01:01*	*DQB1*04:02*	*DQA1*04:01*	0	0	0	0	0
Case 2: DR11,16[b]									
DR1	*DRB1*01:01*	None	*DQB1*05:01*	*DQA1*01:01*	12	8	0	2	2
DR4	*DRB1*04:01*	*DRB4*01:01*	*DQB1*03:01*	*DQA1*03:02*	16	3	8	0	5
DR7	*DRB1*07:01*	*DRB4*01:01*	*DQB1*02:02*	*DQA1*02:01*	28	7	8	7	6
DR8	*DRB1*08:01*	None	*DQB1*04:02*	*DQA1*04:01*	12	4	0	5	3
DR9	*DRB1*09:01*	*DRB4*01:01*	*DQB1*03:03*	*DQA1*03:02*	27	11	8	3	5
DR10	*DRB1*10:01*	None	*DQB1*05:01*	*DQA1*01:01*	12	8	0	2	2
DR11	*DRB1*11:01*	*DRB3*02:02*	*DQB1*03:01*	*DQA1*05:01*	0	0	0	0	0
DR12	*DRB1*12:01*	*DRB3*02:02*	*DQB1*03:01*	*DQA1*05:01*	6	6	0	0	0
DR13	*DRB1*13:01*	*DRB3*01:01*	*DQB1*06:03*	*DQA1*01:03*	20	3	7	8	2
DR14	*DRB1*14:01*	*DRB3*02:02*	*DQB1*05:03*	*DQA1*01:04*	13	8	0	2	3
DR15	*DRB1*15:01*	*DRB5*01:01*	*DQB1*06:02*	*DQA1*01:02*	9	0	2	7	0
DR16	*DRB1*16:02*	*DRB3*02:02*	*DQB1*03:01*	*DQA1*05:01*	2	2	0	0	0
DR17	*DRB1*03:01*	*DRB3*01:01*	*DQB1*02:01*	*DQA1*05:01*	19	5	7	7	0
DR18	*DRB1*03:02*	*DRB3*01:01*	*DQB1*04:02*	*DQA1*04:01*	18	3	7	5	3

[a]Recipient DR15: *DRB1*15:01, DRB5*01:01, DQB1*05:02, DQA1*01:02*; Type DR18: *DRB1*03:02, DRB3*01:01, DQB1*04:02, DQA1*04:01*.
[b]Recipient DR11: *DRB1*11:01, DRB3*02:01, DQB1*03:01, DQA1*05:01*; Type DR16: *DRB1*16:01, DRB5*02:02, DQB1*05:02, DQA1*01:02*.

the Collaborative Transplant Database showed no significant association between triplet matching and kidney graft survival (16), these data showed clearly similar 5-year graft survivals for the zero-antigen mismatches and groups with zero or few triplet mismatches (17). Interestingly, Opelz and coworkers (18) have reported that mismatching for HLA-DP, especially at the level of structurally defined DPB epitopes, had an adverse effect on kidney transplant survival.

Corneal transplants

Class I triplet-based matching is also associated with a better prognosis of penetrating keratoplasty and reduces the time on the waiting list for most patients awaiting a corneal transplant (19, 20). Böhringer et al. (21) recently described an HLAMatchmaker-based tolerogenicity formulation determined from the numbers of mismatched HLA-A,-B and HLA-DR eplets. They determined empirically the optimal thresholds of favorable tolerogenic factors calculated as (# Mm DR eplets)^2 − (# Mm A,B eplets)^2 as less than 220 and fewer than 10 mismatched HLA-A,-B eplets in a cohort of 586 normal-risk patients. In a second cohort of 975 consecutive low-risk and high-risk penetrating keratoplasties, these threshold estimates correlated with better rejection-free survival rates. The authors suggest the usefulness of HLAMatchmaker and that operational tolerance might be inducible by balanced matching of HLA-class I and II eplets (21).

Hematopoietic cell transplants

It is tempting to consider HLAMatchmaker for identifying stem cell donors with permissible mismatches. This algorithm is based, however, on structural configurations reacting with alloantibodies rather than alloreactive T-lymphocytes which play a major role in stem cell transplant failures. Moreover, triplet matching does not predict *in vitro* alloreactive cytotoxic T-cell responses (22). A National Marrow Donor Program study of 744 unrelated hematopoietic cell transplantation cases with one HLA-A,-B, or -C mismatch and 1690 fully HLA-A,-B,-C,-DR,-DQ allele matched cases has shown that class I triplet matching had a very modest effect on engraftment and reduced graft *vs* host disease (23). In multivariate models adjusting for other significant clinical risk factors,

the degree of triplet mismatching did not significantly correlate with patient survival, engraftment, or acute graft *vs* host disease. Other structurally based strategies could be pursued to identify permissible HLA mismatches in hematopoietic cell transplantation. Because transplant outcome is primarily affected by cellular immune mechanisms mediated by various types of T-lymphocytes and natural killer cells, a structural matching algorithm must take into account all amino acid polymorphisms of HLA including the residues in the peptide-binding groove that are important in determining the repertoire of HLA bound peptides, the amino acids on the α1 and α2 helices that contact the T-cell receptors and the polymorphisms that affect natural killer activity. With this complexity, the development of a permissible HLA mismatch strategy for stem cell transplantation represents a considerable challenge.

HLA epitope load and platelet transfusion

Alloimmunization against HLA class I antigens is a major cause of refractoriness of thrombocytopenic patients to random donor platelet transfusions. Although the application of leukoreduction to platelet and red cell preparations has markedly lowered the frequency of immune refractoriness, significant proportions of thrombocytopenic patients become allosensitized and require HLA compatible platelets. During the past three decades HLA matching for platelet transfusion of refractory thrombocytopenic patients has been based on serologic cross-reactivity between HLA-A and HLA-B (24, 25). These so-called B1X, B1U, B2X, etc., match categories are still widely implemented by many blood banks but often enough, this matching strategy is ineffective. An investigation at the National Institutes of Health Clinical Center first showed the potential of HLAMatchmaker in refining and expanding platelet donor selection for refractory, thrombocytopenic patients (26). Another study has also shown that eplet matching benefits platelet transfusions and makes better use of a limited donor pool (27).

An HLA epitope-based matching protocol may lead to a more effective platelet transfusion management (28). This protocol includes high-resolution HLA-A, -B, -C typing of patients and donors, serum screening to identify acceptable mismatches, and the identification of suitable donors in a donor database that incorporates HLAMatchmaker as a search engine.

HLAMatchmaker and diagnostic applications

Serum analysis for HLA epitope-specific antibodies

The analysis of serum reactivity of transplant patients has two goals. Most commonly used is the identification of unacceptable HLA antigens that should be avoided in donor organs. This system is designed to identify donors who must be excluded, but it does not necessarily mean that all other HLA antigens would be compatible for a patient. The other goal is to determine HLA antigens that are acceptable mismatches. This strategy represents a direct approach of finding a compatible donor for a sensitized patient (29, 30).

HLAMatchmaker has been useful in the analysis of antibody reactivity patterns with HLA panels. Often enough, a mismatched antigen can induce antibodies that also react with other antigens that share epitopes with the immunizing antigen. Ignoring such shared epitopes may lead to erroneous interpretations about the distinction between donor-specific and third-party antibodies. It should be noted that HLA antibody responses are generally restricted to a limited number of epitopes (31–33). Highly sensitized patients also have a limited number of antibody specificities but some of them are directed to high-frequency epitopes shared by multiple antigens in the panel. The terms ‘polyspecific’ or ‘multispecific’ seem to reflect a lack of understanding of the sensitization status and this could be avoided by an epitope specificity analysis of highly reactive sera.

The HLAMatchmaker-based analysis of serum reactivity incorporates patient’s HLA type determined preferably by DNA methods at the four-digit allele level. HLA information of the immunizer (for instance, a previous transplant) will identify structurally defined epitopes the patient has been exposed to. This facilitates the interpretation of serum screening results and the determination of mismatch acceptability for prospective donors.

Several antibody analysis programs for solid-phase assays with single HLA kits can be downloaded at no charge from the website www.HLAMatchmaker.net. These programs are on MICROSOFT EXCEL sheets on which one can enter the four-digit HLA types of antibody producer and immunizer as well as the HLA types of the panel and the reaction scores. The first step is to record into the program those alleles that give negative reactions with patient’s serum. Such alleles can be expected to have epitopes that are not recognized by patient’s antibodies and the program automatically removes these epitopes displayed on all alleles in the panel. The remaining eplets on the reactive alleles can be sorted to determine antibody-reactive eplets including those shared with immunizing alleles. Although commercial assay kits only contain the more common alleles, HLAMatchmaker can also identify antibody-reactive eplets on other alleles not included in these kits.

The application of HLAMatchmaker to the antibody analysis may increase our understanding of an otherwise unexplained sensitization pattern induced a given mismatch. For instance, we have recently reported a case whereby a transplant recipient who typed as *B*44:03* had antibodies that reacted with *B*44:02* (11). This patient was sensitized by the donor’s *C*07:04* which carries the 156DA eplet shared with *B*44:02* and a group of HLA-B alleles including *B*08:01*, *B*37:01*, *B*41:01*, *B*42:01*, *B*44:02*, *B*45:01*, and *B*82:01*, all of them reacted with patient’s serum. In another case,

a 151ARE mismatch on a *C*02:02* mismatch had induced antibodies that reacted with all 151ARE-carrying HLA-B alleles (34). These findings show that sensitization induced by an epitope on an HLA-C mismatch may cause other class I antigens to become unacceptable mismatches because they share that epitope although the patient may have never been exposed to such antigens.

HLAMatchmaker can also explain unexpected reactivity patterns of class II antibodies. For instance, patients sensitized by a DR2 mismatch may have antibodies that react also with DR1 (35). Although these antigens might share a distinct epitope recognized by these antibodies, HLAMatchmaker cannot predict such structurally defined epitope. Nevertheless, the reactivity with DR1 can be readily explained with antibodies induced by DR51 which is in strong linkage disequilibrium with DR2. They are specific for the 96EV eplet shared between DR51 and DR1. Conversely, sensitization by a DR1 mismatch can lead to antibodies that react also with DR51 but not with DR2. These findings show that 96EV represents a highly immunogenic epitope that can induce cross-sensitization between antigens encoded by different DRB loci. Another report has shown the importance of DRB3/4/5 eplets in DRB-specific antibody responses of kidney transplant recipients (12). This study indicated that donor-specific antibody responses were more frequent to DRB3/4/5 mismatches than to DRB1 mismatches and this correlated with eplet loads. We also noted higher frequencies of donor-specific DQB and DQA antibodies and these mismatches had higher eplet loads than the DRB1 mismatches.

HLAMatchmaker is also a useful algorithm to analyze the reactivity patterns of anti-MICA antibodies and the determination of MICA mismatch acceptability at the structural level alloantibodies (6). Molecular viewing of the MICA structure and the determination of amino acid sequence differences between MICA alleles have yielded a repertoire of 38 potentially immunogenic MICA eplets. Analyses of MICA-reactive sera are often specific for supereplets CMGWS (combination of 36C, 129M, 206GW, and 215S) and AYVE (combination of 25AY, 129V, and 173E) as well as the 25TC, 14W, 14G, and 122V eplets.

HLAMatchmaker and the management of highly sensitized patients

Permissible and immunogenic HLA antigens can be determined from epitope analyses of HLA-specific antibodies in sensitized patients (36). HLAMatchmaker is a useful tool in the analysis of serum antibody reactivity of sensitized patients and the identification of potential donors with acceptable mismatches (8, 30, 37–39). Eurotransplant has incorporated HLAMatchmaker in the Acceptable Mismatch Program to identify donors for highly sensitized patients (30, 38, 39). This approach shortens the waiting time for a suitable kidney donor and leads to excellent graft survivals comparable to those seen with non-sensitized recipients (30).

Other investigators have also reported the usefulness of HLAMatchmaker in the clinical setting. Investigators in Greece showed that patients with rejected transplants had antibodies specific for triplets on donor class I mismatches and they suggested that this information is beneficial in the selection of suitable donors for retransplantation (40, 41). Goodman et al. (8) reported a strong correlation between the number of mismatched triplets and the presence of HLA antibodies detected in Luminex assays with single class I alleles. Valentini et al. applied HLAMatchmaker to the selection of successful kidney transplants for two highly sensitized pediatric patients treated with intravenous immunoglobulin (Ig) (42, 43).

How do eplets compare to antibody-defined HLA epitopes?

Although HLAMatchmaker is a theoretical model that predicts eplets as critical components of HLA epitopes, the question remains how many eplets correspond to epitopes specifically recognized by antibodies. We have performed a comparative analysis of how antibody-defined epitopes described by Terasaki and coworkers (44–46) correspond to HLAMatchmaker-defined eplets (47, 48). Table 3 lists examples of class I eplets that are equivalent to antibody-defined epitopes and their molecular locations are depicted in

Table 3 Examples of eplets that correspond to antibody-verified epitopes reported by Terasaki and coworkers

Eplet	TerEp	Antibody-reactive antigens	Models
127K	#19	A2,23,24,68,69	Figure 1A
144KR	#208	A1,3,11,24,36,80	Figure 1A
65GKA	#3	A23,24	Figure 1A
62EE	#28	A23,24,80	Figure 1A
167DG	#14	A1,23,2402,80, B76	Figure 1A
82LR	#24	A23,24,25,32, B13,2705,37,38,44,47,49,51,52, 53,57,58,59,63,77	Figure 1A, B
79RI	#23	A23,24,25,32, B38,49,51, 52,53,57,58,59,63,77	Figure 1A, B
44RT	#35	B18,35,37,51,52,53,58,78	Figure 1B
71NT	#22	B8,13,18,35,37,38,39,3905,4005, 41,44,45,47,48,49,50,51,52,53, 59,60,61,62,64,65,71,72,75,76, 77,78	Figure 1B
163LW	#245	B35,4005,46,49,50,51,52,53,56, 57,58,62,63,71,72,75,77,78, Cw9,w10	Figure 1B, C
21H	#39	Cw2,w9,w10,w15	Figure 1C
80VRN	#246	B46,73, Cw1,w7,w8,w9,w10,w12, w14,w16	Figure 1C
77TVS	#421	B46, Cw1,w8,w9,w10,w14,w16	Figure 1C

Table 4 Examples of eplet pairs that correspond to epitopes recognized by human monoclonal antibodies

mAb name	HLA-type antibody producer	Immunizer	Eplet specificity	Distance (Å)	Reactive antigens	Self-eplet on	Models
MUL4C8	A2,25, B18,51, Cw12,15	A11	144KR+s151H	7	A1,A3,A11,A24,A36	A2,25	Figure 2A
WK1D12	A1,-; B8,-; Cw7,-	B27	163EW+s73TE	16	B7,B13,B27,B40,B47, B48,B81	B8	Figure 2B
HDG11G12	A2,24, B7,60, Cw7,w10	B35	163LW+s62RQI	8	B15,B35,B49,B50,B51, B52,B53,B56,B78	B7,60	Figure 2C

Figure 1. About one-half of Terasaki's class I epitopes correspond to eplets and about one-third of them are equivalent to pairs of eplets. The latter is analogous to our findings with human monoclonal antibodies specific for epitopes defined by non-self-eplets paired with self-eplets separated far enough for contact by two different complementarity-determining regions (CDRs) of antibody (2, 49). A recent report describes 10 human monoclonal antibody-reactive epitopes defined by eplet pairs (50). Table 4 lists three examples of eplet pairs shared by the immunizing antigen and the reactive antigens in Luminex single allele panels. As illustrated in Figure 2, the non-self-eplets and self-eplets in these pairs are between 7 and 16 Å apart. Apparently, these self-eplets function as critical contact sites with antibody. Antigens that have the same non-self-eplet but lack the critical self-eplet do not react with antibody.

Another report shows that most of Terasaki's HLA-DR and DQ epitopes are equivalent to eplets and less than 10% of them corresponded to DRB eplet pairs (48). Studies by Tambur and coworkers (51, 52) have shown that about one-third of anti-DQ antibodies in kidney transplant candidates are directed against epitopes with amino acid configurations shared between non-self-DQB chains paired with self-DQA chains or vice versa. Such epitopes are generated by combinations of αβ chains of DQ heterodimers. HLAMatchmaker analysis showed as an example a DQ epitope defined by 74NSL on DQA and 56PPD on DQB. These eplets are about 9 Å apart.

How do eplet pairs constitute distinct epitopes? This question can be addressed with current concepts about the structure of the antigen–antibody interface. Stereochemical analyses of crystallized antigen–antibody complexes have defined a structural epitope as that part of the antigen that makes contact with the six CDR loops of antibody. A structural epitope has about 15–25 contact residues with a surface area of 700–900 $Å^2$ (53–55). The binding or functional activity of an epitope depends on a small subset of energetic residues in the so-called hot spot locations in the structural epitope and contacted by antibody (56–60). In order to be immunogenic, a functional epitope must have at least one non-self-residue, i.e. the antibody producer's homologous proteins must have a different residue in the corresponding sequence position. Such residues must be on the molecular surface so they can make contact with the specificity-determining CDRs of antibody. Although the third CDR of the Ig heavy chain (CDR-H3) often plays a dominant role, other CDRs with energetic residues may provide important contacts with epitope (54).

On the basis of a more or less circularly sized surface area of 700–900 $Å^2$, one can calculate that the contact residues of a structural epitope can be maximally about 15 Å away from a centrally located residue as a component of the functional epitope. In comparison, HLA molecules seen from the top (i.e. including both helices and bound peptide) have surface areas of about 750 $Å^2$. In other words, HLA alloantibodies can make contact with a rather substantial part of the HLA molecular

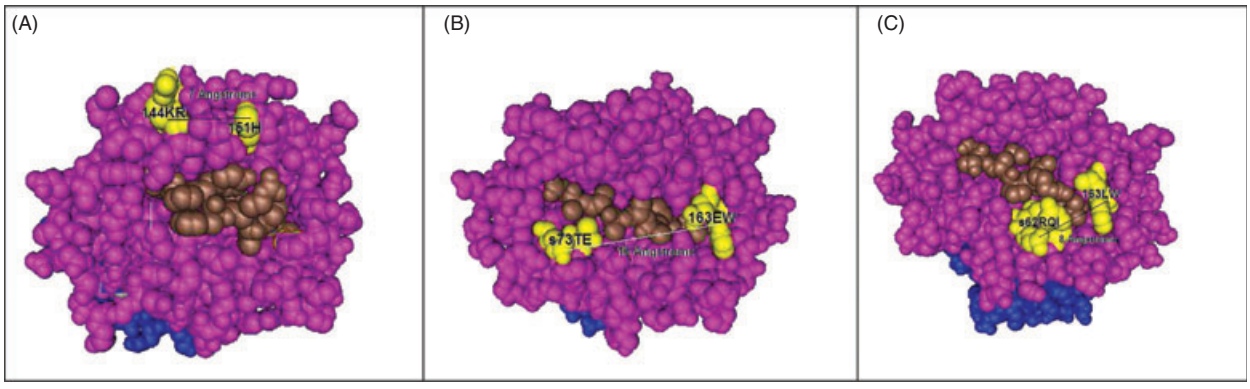

Figure 2 Examples of molecular locations of class I eplet pairs that correspond to antibody-verified epitopes (Table 4). (A) 144KR+s151H on HLA-A11, (B) 163EW + s73TE on HLA-B27, and (C) 163LW + s62RQI on HLA-B35.

surface, but the binding strength is determined primarily by mismatched eplets in selected locations of structural epitopes. Mismatched eplets interact with the specificity-determining CDRs, whereas self-eplets serve as critical contact sites with other CDRs of antibody. The latter would be important for the formation of a stable antigen–antibody complex.

It seems therefore that alloantibody responses to HLA mismatches may have an autoreactive component. This postulate might be viewed in context with current concepts of antibody structure and B-cell diversity (61). During B-cell development, rearrangements of various Ig heavy (V_H) and light (V_L) genes produce a vast repertoire of Ig receptors with different antigenic specificities including those for self-epitopes on normal tissue proteins. The latter dominate in the primary repertoire (62, 63), but developing B-cells are also programmed to generate negative signals that lead to clonal deletion of many autoreactive cells. However, autoreactive immature B-cells undergo additional successive V_L gene rearrangements referred to as receptor editing (64–66). This leads to B-cell receptors that have lost some of their self-reactivity but they may recognize altered forms of proteins with amino acid substitutions and this would ultimately lead to antibody production (67, 68). This condition may apply to the alloantibody response to HLA antigens.

It is possible that HLA antibody-producing cells originally had B-cell receptors with CDR loops that can interact with self-HLA constituents, whereas other CDR loops are the result of receptor editing or some other gene rearrangement process to accommodate the recognition of non-self-amino acid configurations on mismatched HLA antigens. This concept explains the antibody specificity against HLA epitopes defined by a pair of non-self and self-eplets. It may increase our understanding of epitope immunogenicity and the antibody response patterns to HLA mismatches which seem enhanced by self-recognition. Antibody reactivity to eplet pairs also raises questions about determining mismatch acceptability for sensitized patients. If the patient has antibodies against a mismatched eplet paired with self-configuration, can we consider an eplet-carrying allele as an acceptable mismatch if it lacks this self-configuration?

These findings provide a new insight of HLA immunogenicity. Some antibodies react with all antigens carrying a mismatched eplet originally presented by the immunizing antigen. Other antibodies recognize a mismatched eplet but only in context with a self-configuration in a polymorphic sequence location. We can therefore conclude that a given mismatched eplet can induce specific antibodies with different reactivity patterns that depend on the binding to certain self-configurations shared by reactive alleles. This concept requires modifications of the HLAMatchmaker programs to analyze antibody reactivity with HLA panels. The new versions to be posted on the www.HLAMatchmaker.net website will include potential epitopes described by eplets paired with other eplets located no more than 15 Å away within the dimensions of a structural epitope. Antibody-reactive pairs will be considered in the epitope notation system and the design of the HLA Epitope Database.

Development of an HLA epitope database

In the clinical setting of transplantation, it has become apparent that HLA epitopes rather than antigens are important for matching and analyzing antibody specificity. This notion is particularly relevant to the determination of mismatch acceptability for sensitized patients especially if a potential donor who types for alleles not included in the antigen panel is used for antibody screening. Although many antibody-reactive epitopes have been characterized structurally, it remains to be seen which ones are clinically relevant and how many more epitopes will be identified. Considering the importance of epitope matching in transplantation, there is a need to establish a database for clinically relevant epitopes. A 16th International Histocompatibility Workshop project on an HLA Epitope Database is underway to address this issue.

Participants in this project will look for antibodies specific for newly defined epitopes and amino acid polymorphisms in molecular structure models will be used to describe such epitopes. Information about the immunizing event, such as the HLA differences between immunizer and antibody producer, will enhance the characterization of antibody-defined epitopes. A worldwide effort will likely generate new insights into HLA epitope structure. Altogether, the data from participants together with already existing information will be incorporated into an epitope database that might be useful in HLA matching strategies for clinical transplantation.

Concluding remarks

HLAMatchmaker is based on a structural characterization of HLA epitopes important in alloantibody responses. This algorithm has become a useful tool to determine histocompatibility at the epitope level and permits the identification of acceptable mismatches for sensitized patients as well as an assessment of mismatch permissibility for non-sensitized patients.

References

1. Duquesnoy RJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm. *Hum Immunol* 2002: **63**: 339–52.
2. Duquesnoy RJ. A structurally based approach to determine HLA compatibility at the humoral immune level. *Hum Immunol* 2006: **67**: 847–62.
3. Duquesnoy R. Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. *Curr Opin Immunol* 2008: **20**: 594–601.
4. Duquesnoy RJ, Marrari M. HLAMatchmaker-based definition of structural HLA epitopes detected by alloantibodies. *Curr Opin Organ Transpl* 2009: **14**: 403–9.

5. Duquesnoy RJ, Askar M. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. V. Eplet matching for HLA-DR, HLA-DQ and HLA-DP. *Hum Immunol* 2007: **68**: 12–25.
6. Duquesnoy R, Mostecki J, Hariharan J, Balasz I. A structurally based epitope analysis of MICA antibody specificity patterns. *Hum Immunol* 2008: **69**: 826–32.
7. Dankers MKA, Witvliet MD, Roelen DL et al. The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched HLA antigens. *Transplantation* 2004: **128**: 1236–9.
8. Goodman R, Taylor C, O'Rourke C, Lynch A, Bradley A, Key K. Utility of HLAMatchmaker and single-antigen HLA-antibody detection beads for identification of acceptable mismatches in highly sensitised patients awaiting kidney transplantation. *Transplantation* 2006: **81**: 1331–6.
9. Mihaylova A, Baltadjieva D, Boneva P et al. Clinical relevance of anti-HLA antibodies detected by flow-cytometry bead-based assays – single-center experience. *Hum Immunol* 2006: **67**: 787–94.
10. Kosmoliaptsis V, Bradley J, Sharples L et al. Predicting the immunogenicity of human leukocyte antigen class I alloantigens using structural epitope analysis determined by HLAMatchmaker. *Transplantation* 2008: **85**: 1817–25.
11. Lomago J, Jelenik L, Zern D et al. How did a patient who types for HLA-B*4403 develop antibodies that react with HLA-B*4402? *Hum Immunol* 2010: **71**: 176–8.
12. Duquesnoy R, Awadalla Y, Lomago J et al. Retransplant candidates have donor-specific antibodies that react with structurally defined HLA-DR,DQ,DP epitopes. *Transpl Immunol* 2008: **18**: 352–60.
13. Duquesnoy RJ, Takemoto S, De Lange P, Doxiadis IIN, Schreuder GMT, Claas FHJ. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. III. Effect of matching at the HLA-A,B amino acid triplet level on kidney transplant survival. *Transplantation* 2003: **75**: 884–9.
14. Duquesnoy RJ, Howe J, Takemoto S. HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. IV. An alternative strategy to increase the number of compatible donors for highly sensitized patients. *Transplantation* 2003: **75**: 889–97.
15. Haririan A, Fagoaga O, Daneshvar H et al. Predictive value of HLA epitope matching using HLAMatchmaker for graft outcomes in a predominantly African-American renal transplant cohort. *Clin Transplant* 2006: **20**: 226–33.
16. Laux G, Mytilineos J, Opelz G. Critical evaluation of the amino acid triplet-epitope matching concept in cadaver kidney transplantation. *Transplantation* 2004: **77**: 902–7.
17. Duquesnoy R, Claas F. Is the application of HLAMatchmaker relevant in kidney transplantation? (Letter to the Editor). *Transplantation* 2005: **79**: 250–1.
18. Laux G, Mansmann U, Deufel A, Opelz G. A new epitope-based HLA-DP matching approach for cadaveric kidney transplantation. *Transplantation* 2003: **75**: 1527–32.
19. Boehringer D, Reinhard T, Duquesnoy R et al. Beneficial effect of matching at the HLA-A and B amino-acid triplet level on rejection free survival in penetrating keratoplasty. *Transplantation* 2004: **77**: 417–21.
20. Böhringer D, Reinhard T. Praktikabilität des HLA-matchings. *Ophthalmologe* 2007: **104**: 219–21.
21. Böhringer D, Daub F, Schwartzkopff J et al. Operational post-keratopasty graft tolerance due to differential HLAMatchmaker matching. *Mol Vis* 2010: **16**: 2362–7.
22. Dankers MKA, Heemskerk MHM, Duquesnoy RJ et al. HLAMatchmaker algorithm is not a suitable tool predict the alloreactive cytotoxic T lymphocyte response *in vitro*. *Transplantation* 2004: **78**: 165–71.
23. Duquesnoy RJ, Haagenson M, Spellman S, Wang T, Oudshoorn M. Triplet matching is not associated with better survival rates of patients with class I HLA allele mismatched hematopoietic cell transplants from unrelated donors. *Biol Blood Marrow Transplant* 2008: **14**: 1064–71.
24. Duquesnoy RJ, Filip DJ, Rodey GE, Rimm AA, Aster RH. Successful transfusion of platelets "mismatched" for HLA antigens to alloimmunized thrombocytopenic patients. *Am J Hematol* 1977: **2**: 219–26.
25. Brecher ME. *Technical Manual*. *Bethesda*: *American Association of Blood Banks*, 2005.
26. Nambiar A, Duquesnoy RJ, Adams S et al. HLAMatchmaker-driven analysis of response to HLA matched platelet transfusions in alloimmunized patients. *Blood* 2006: **107**: 1680–7.
27. Brooks E, MacPherson B, Fung M. Validation of HLAMatchmaker algorithm in identifying acceptable HLA mismatches for thrombocytopenic patients refractory to platelet transfusions. *Transfusion* 2008: **48**: 2159–66.
28. Duquesnoy R. Structural epitope matching for HLA alloimmunized thrombocytopenic patients: a new strategy to provide more effective platelet transfusion support? *Transfusion* 2008: **I48**: 221–7.
29. Claas FH, Gijbels Y, von Veen A et al. Selection of cross-match negative HLA-A and/or -B mismatched donors for highly sensitized patients. *Transplant Proc* 1989: **21**: 665–6.
30. Claas FHJ, Witvliet M, Duquesnoy RJ, Persijn G, Doxiadis IIN. The acceptable mismatch program as a fast tool to transplant highly sensitized patients awaiting a post-mortal kidney: short waiting time and excellent graft outcome. *Transplantation* 2004: **78**: 190–3.
31. Duquesnoy RJ, White LT, Fierst JW et al. Multiscreen serum analysis of highly sensitized renal dialysis patients for antibodies toward public and private class I HLA determinants. Implications for computer-predicted acceptable and unacceptable donor mismatches in kidney transplantation. *Transplantation* 1990: **50**: 427–37.
32. Rodey GE, Revels K, Fuller TC. Epitope specificity of HLA class I alloantibodies. II. Stability of cross-reactive group antibody patterns over extended time periods. *Transplantation* 1997: **63**: 885–93.
33. Papassavas AC, Iniotaki-Theodoraki A, Boletis J, Douramani P, Kostakis A, Stavropoulos-Giokas C. A limited number of HLA epitopes are recognized from HLA class I-specific antibodies detected in the serum of sensitized patients. *Hum Immunol* 2000: **61**: 705–10.
34. Duquesnoy R, Marrari M. Detection of antibodies against HLA-C epitopes in patients with rejected kidney transplants. *Transplant Immunol* 2011 (in press).

35. Marrari M, Duquesnoy R. Why can sensitization by a HLA-DR2 mismatch lead to antibodies that react also with HLA-DR1? *Hum Immunol* 2009: **70**: 403–9.
36. Papassavas AC, Stavropoulos-Giokas C, Boletis J, Ioannou S, Iniotaki-Theodoraki A, Kostakis A. Definition of permissible and immunogenic HLA antigens based on epitope analysis of the HLA specific antibodies produced in sensitized patients. *Eur J Immunogenet* 2002: **29**: 401–7.
37. Duquesnoy RJ, Witvliet MJ, Doxiadis IIN, de Fijter H, Claas FHJ. HLAMatchmaker-based strategy to identify acceptable HLA class I mismatches for highly sensitized kidney transplant candidates. *Transpl Int* 2004: **7**: 31–8.
38. Claas FHJ, Dankers MK, Oudshoorn M et al. Differential immunogenicity of HLA mismatches in clinical transplantation. *Transpl Immunol* 2005: **14**: 187–91.
39. Doxiadis IIN, Duquesnoy RJ, Claas FHJ. Extending options for highly sensitized patients to receive a suitable kidney graft. *Curr Opin Immunol* 2005: **17**: 536–40.
40. Iniotaki-Theodoraki A, Kalogeropoulou E, Apostolaki M, Doxiadis I, Stavropoulos-Giokas C. Humoral sensitization against rejected grafts: specific antibodies to graft immunogenic amino acid triplets. *Transplant Proc* 2004: **36**: 1728–31.
41. Varnavidou-Nicolaidou A, Doxiadis II, Iniotaki-Theodoraki A et al. HLA class I donor-specific triplet antibodies detected after renal transplantation. *Transplant Proc* 2004: **36**: 1732–4.
42. Valentini RP, Nehlsen-Cannarella SL, Gruber SA et al. Intravenous immunoglubulin, HLA allele typing and HLAMatchmaker facilitate successful transplantation in highly sensitized pediatric renal allograft recipients. *Pediatr Transplant* 2007: **11**: 77–81.
43. Duquesnoy RJ. "Match and Treat", an effective strategy for transplanting highly sensitized pediatric transplant candidates? (Invited Editorial). *Pediatr Transplant* 2007: **11**: 3–4.
44. El-Awar NR, Akaza T, Terasaki PI et al. Human leukocyte antigen class I epitopes: update to 103 total epitopes, including the C locus. *Transplantation* 2007: **84**: 532–40.
45. Cai J, Terasaki PI, Mao Q et al. Development of nondonor-specific HLA-DR antibodies in allograft recipients is associated with shared epitopes with mismatched donor DR antigens. *Am J Transplant* 2006: **6**: 2947–54.
46. Deng CT, El-Awar N, Ozawa M et al. Human leukocyte antigen class II DQ alpha and beta epitopes identified from sera of kidney allograft recipients. *Transplantation* 2008: **86**: 452–9.
47. Duquesnoy R, Marrari M. Correlations between Terasaki's HLA class I epitopes and HLAMatchmaker-defined eplets on HLA-A, -B and -C antigens. *Tissue Antigens* 2009: **74**: 117–33.
48. Marrari M, Duquesnoy R. Correlations between Terasaki's HLA class II epitopes and HLAMatchmaker-defined eplets on HLA-DR, -DQ and -DP antigens. *Tissue Antigens* 2009: **74**: 134–46.
49. Adeyi OE, Girnita A, Awadalla Y et al. Serum analysis after kidney transplant nephrectomy reveals restricted antibody specificity patterns against donor HLA class I antigens. *Transpl Immunol* 2005: **14**: 53–62.
50. Marrari M, Mostecki J, Mulder A, Balazs I, Claas F, Duquesnoy R. Human monoclonal antibody reactivity with HLA class I epitopes defined by pairs of mismatched eplets and self eplets. *Transplantation* 2010: **90**: 1468–72.
51. Barabanova Y, Ramon D, Tambur AR. Antibodies against HLA-DQ alpha-chain and their role in organ transplantation. *Hum Immunol* 2009: **70**: 410–8.
52. Tambur A, Leventhal JR, Friedewald J, Ramon D. The complexity of human leukocyte antigen (HLA)-DQ antibodies and its effect on virtual crossmatching. *Transplantation* 2010: **90**: 1117–24.
53. Davies D, Padlan E, Sheriff S. Antibody-antigen complexes. *Annu Rev Biochem* 1990: **59**: 439–73.
54. MacCallum RM, Martin ACR, Thornton JM. Antibody-antigen interactions: contact analysis and binding site topography. *J Mol Biol* 1996: **262**: 732–45.
55. Padlan EA. Anatomy of the antibody molecule. *Mol Immunol* 1994: **31**: 169–217.
56. Getzoff ED, Tainer JA, Lerner RA, Geysen HM. The chemistry and mechanism of antibody binding to protein antigens. *Adv Immunol* 1988: **43**: 1–98.
57. Novotny J. Protein antigenicity: a thermodynamic approach. *Mol Immunol* 1991: **28**: 201–8.
58. Laune D, Molina K, Ferrieres G et al. Systematic exploration of the antigen binding activity of synthetic peptides isolated from the variable regions of immunoglobulins. *J Biol Chem* 1997: **272**: 30937–44.
59. Bogan AA, Thorn KS. Anatomy of hot spots in protein interfaces. *J Mol Biol* 1998: **280**: 1–9.
60. Van Regenmortel M. Reductionism and the search for structure-function relationships in antibody molecules. *J Mol Recognit* 2002: **15**: 240–7.
61. Murphy K, Travers P, Walport M, Janeway C. *Janeway's Immunobiology*, *7th edn*. *New York*: *Garland Science*, *2008*.
62. Gaudin E, Rosado M, Agenes F, McLean A, Freitas A. B-cell homeostasis, competition, resources, and positive selection by self-antigens. *Immunol Rev* 2004: **197**: 102–20.
63. Cancro M, Kearney J. B cell positive selection: road map to the primary repertoire. *J Immunol* 2004: **173**: 15–9.
64. Gay D, Saunders T, Camper S, Weigert M. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J Exp Med* 1993: **177**: 999–1006.
65. Tiegs S, Russell D, Nemazee D. Receptor editing in self-reactive bone marrow B cells. *J Exp Med* 1993: **177**: 1009–17.
66. Reiter M, Nemazee D. Receptor editing occurs frequently during normal B cell development. *J Exp Med* 1998: **188**: 1231–8.
67. Tze L, Baness E, Hippen K, Behrens T. Ig light chain receptor editing in anergic B cells. *J Immunol* 2000: **165**: 6796–802.
68. Wardemann H, Hammersen J, Nussenzweig M. Human autoantibody silencing by immunoglobulin light chains. *J Exp Med* 2004: **200**: 191–9.

