Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Immunology

Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation

René J Duquesnoy

HLAMatchmaker is a computer algorithm that determines HLA compatibility at the structural level. Donor–recipient histocompatibility is assessed with polymorphic amino acid configurations that represent structurally defined elements of HLA epitopes originally assigned as triplets and more recently as eplets. For many patients, HLAMatchmaker can identify mismatched HLA antigens that can be considered compatible at the structural level. Structurally based HLA matching reduces humoral allosensitization and correlates with good transplant outcome. Moreover, HLAMatchmaker is useful in the analysis of serum antibody reactivity and benefits the strategy of identifying acceptable mismatches for highly sensitized patients.

Addresses
Division of Transplantation Pathology, University of Pittsburgh Medical Center, Thomas E. Starzl Biomedical Science Tower, Room W1552, Pittsburgh, PA 15261, United States

Corresponding author: Duquesnoy, René J (Duquesnoyr@upmc.edu)

Current Opinion in Immunology 2008, **20**:594–601

This review comes from a themed issue on
Immunogenetics and Transplantation
Edited by Frans H. J. Claas and Rene Duquesnoy

Available online 7th August 2008

0952-7915/$ – see front matter

DOI 10.1016/j.coi.2008.06.010

Introduction

In organ transplantation, the degree of HLA compatibility is generally determined by counting the number of mismatched HLA-A, B, DR antigens of the donor. It is well known that the zero-antigen mismatches have the highest success rates but why do so many mismatched transplants do so well? The answer to this question seems related to the antibody responses to HLA mismatches. Many studies have demonstrated that HLA class I and class II antibodies are significant risk factors for transplant failure.

An important consideration is that HLA antigens have multiple epitopes that can be recognized by specific antibodies. The original description of the epitope repertoire was based on serological cross-reactivity between HLA antigens and antibody specificities against so-called private and public determinants. During the eighties and nineties, many studies with HLA-specific monoclonal antibodies provided evidence that private and public epitopes correspond to distinct amino acid residues or short sequences in HLA. These findings have been applied to the serum antibody analysis of sensitized patients and amino acid residue matching for kidney transplantation. Most recently, Terasaki's group has conducted extensive studies on antibody reactivity patterns with single HLA alleles in antigen-binding assays on a Luminex platform [1–3] As described elsewhere in this issue, these analyses have identified 103 class I and 83 class II epitopes defined by polymorphic amino acid residues [4]. This review describes how HLAMatchmaker approaches HLA compatibility at the epitope level.

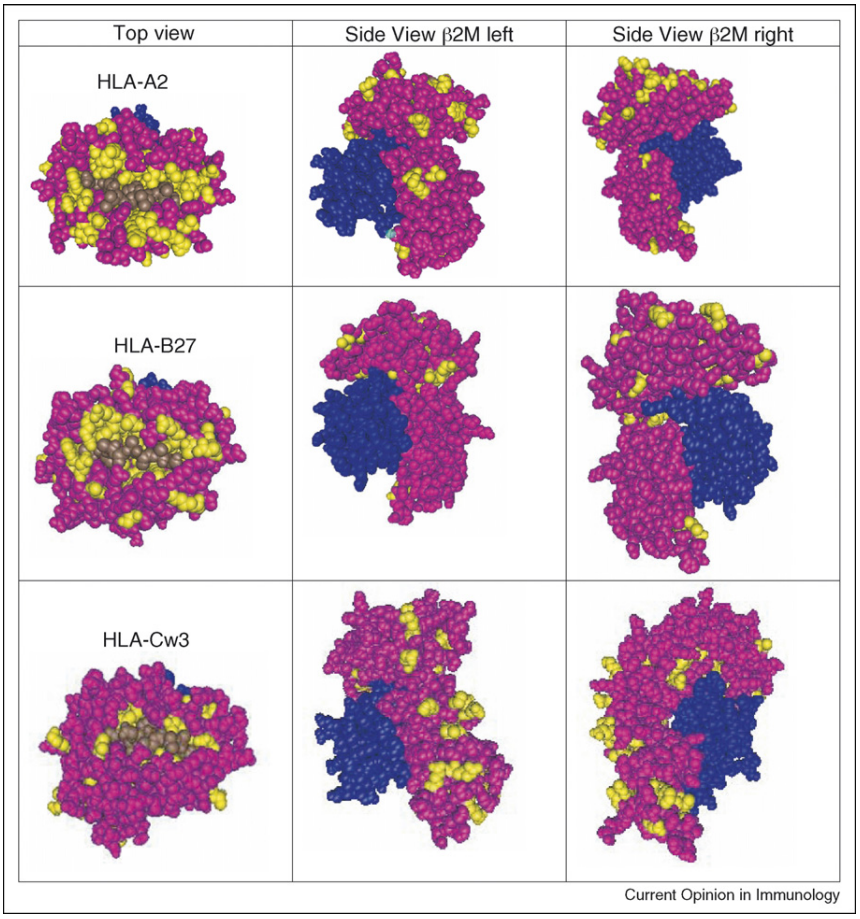
Amino acid residue polymorphisms on the HLA molecular surface

HLA epitopes are determined by antibody-accessible polymorphic amino acid residues on the molecular surface. Three-dimensional modeling of HLA molecules has revealed a broad array of polymorphic residues. Figure 1 shows the polymorphic residues on three crystallized class I molecules, HLA-A2, HLA-B27 and HLA-Cw3. The molecular surface around the bound peptide (see top view) has similar numbers of exposed polymorphic positions on the α1 helices of HLA-A and HLA-B antigens but more polymorphic positions are visible on the α2 helices of HLA-A antigens. The α helices of HLA-C antigens have much fewer polymorphic positions.

By contrast, HLA-C antigens have many polymorphic positions in the membrane-proximal domain. HLA-A antigens have also more surface-exposed polymorphic positions in that region and it should be noted that the sequence positions in the membrane-proximal domain of HLA-B are all monomorphic.

HLA-DR and HLA-DQ molecules have different patterns of surface expression of polymorphic residues (Figure 2). The structural polymorphism of HLA-DR is restricted to the β chain, the α chain is monomorphic. Polymorphic residues are readily visible on the top of the molecule adjacent to the bound peptide and many of them involve contiguous sequences. Polymorphic residues on the side of the molecule generally comprise distinct clusters in both β1 and β2 domains. A few polymorphisms are visible at the bottom part of the molecule nearby the cell membrane. DRB and DQB

Figure 1



Polymorphic residue positions on HLA-A, B and C molecules. The following crystalline models were downloaded from the National Center for Biotechnology Information (NCBI) website http://www.ncbi.nlm.nih.gov/Structure: A*0201 (PDB #1JF1), B*2705 (PDB #1HSA) and Cw*0302 (PDB #1EFX) and viewed with the Cn3D software program [5].

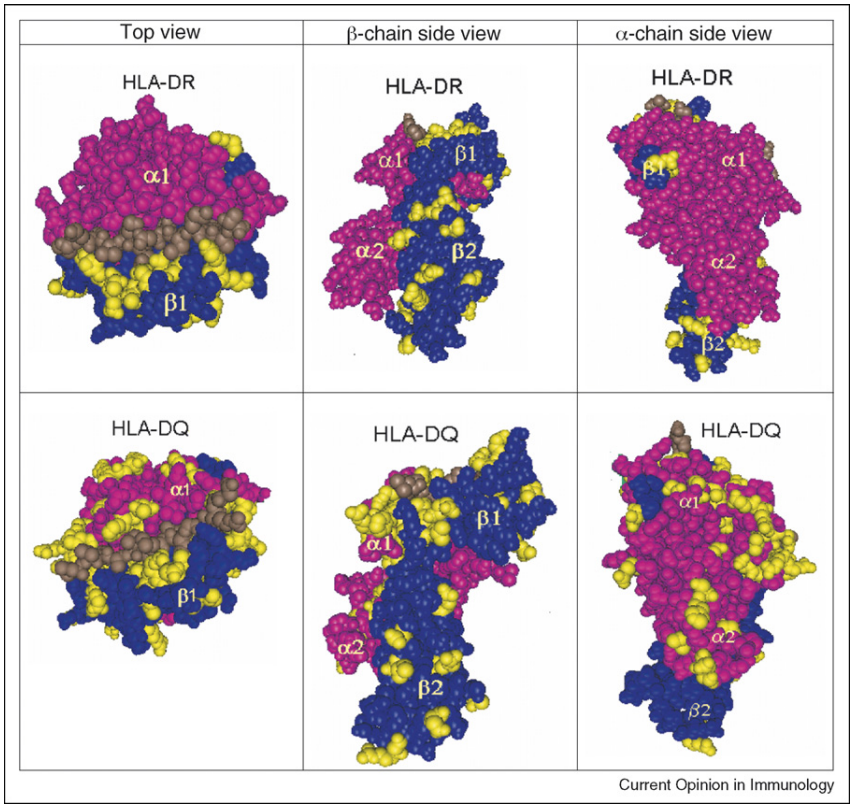
seem to show similar numbers of polymorphic positions. DQA displays somewhat contiguous polymorphic positions on the top of the molecule nearby the bound peptide and on the side of the α1 domain. The polymorphic positions in the α2 domain seem to be more in distinct clusters.

Altogether, these models illustrate the rather extensive degree of structural polymorphism and they suggest that epitopes have a widespread distribution over the molecular surface. Depending on their location, epitopes may have different degrees of antibody accessibility. Some antibodies may react with epitopes on solubilized but not with membrane-bound HLA molecules.

The concept of HLAMatchmaker

HLAMatchmaker was originally introduced as a matching algorithm whereby each HLA antigen is viewed as a string of short linear sequences (triplets) involving polymorphic amino acid residues in antibody-accessible positions; they are considered key elements of epitopes that can induce the formation of specific antibodies [6•]. Although, as shown below, the triplet version of HLA-Matchmaker has proven to be clinically useful, it provides an incomplete description of the structural HLA epitope repertoire. Expanded criteria including longer sequences and polymorphic residues in discontinuous positions have been applied to a new version of HLAMatchmaker developed from stereochemical modeling of crystallized complexes of antibodies with different protein antigens and published data about the about contributions of critical amino acid residues to antigen–antibody binding energy [7••]. Antigenic proteins have structural epitopes consisting of 15–22 residues that constitute the binding face with antibody. The surface of a structural epitope varies between 700 and 850 $Å^2$ and is about the same as the surface around the bound peptide-binding groove of an HLA molecule. Most structural epitopes have a patch

Figure 2



Polymorphic amino residue positions on HLA-DRB and HLA-DQ molecules. The following crystalline models were downloaded from the NCBI website http://www.ncbi.nlm.nih.gov/Structure: DRA1*0101, DRB1*0101 (PDB # 1KG0) and DQA1*0301, DQB1*0302 (PDB # 1JK8) and viewed with the Cn3D software program [5].

of about 2–5 so-called highly energetic residues (sometimes referred to as 'hot spots') that dominate the strength and specificity of binding with antibody. The residues of such functional epitope are about 3 Å apart from each other and at least one of them is non-self. The remaining residues of a structural epitope contribute supplementary interactions that increase the stability of the antigen–antibody complex. These concepts have been applied to the new version of HLAMatchmaker [7••].

Class I HLA molecules have 75 polymorphic positions and a determination of their residue compositions within a 3-Å radius has yielded a total of 199 so-called eplets on HLA-A, B, C antigens, 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 [7••].

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 represents therefore a more complete repertoire of structurally defined HLA epitopes and provides a more detailed assessment of HLA compatibility.

Each eplet is assigned a position number in the amino acid sequence and the polymorphic residues within a radius of about 3 Å; this notation does not use monomorphic residues. Amino acid residues are marked with the standard letter code. For instance, the class I eplet 11AMR is in sequence position 11 and has three polymorphic residues: alanine (A), methionine (M) and arginine (R). Many eplets are listed with one or two residues (for instance 9H and 193PV) because their neighboring residues are the same on all HLA Class I chains and they are therefore not shown.

HLAMatchmaker applies two principles: (1) each HLA antigen represents a distinct string of structurally defined epitopes as potential immunogens that can induce specific antibodies and, (2) patients cannot make antibodies against epitopes that are expressed by their own HLA molecules [6•]. The algorithm assesses donor–recipient compatibility through intralocus and interlocus comparisons, and determines what epitopes on mismatched HLA molecules are

Table 1

Examples of how structurally based compatibility of a mismatched HLA antigen depends on the HLA phenotype of the recipient

Case	Phenotype						#Ep	B51 (B*5101), mismatched eplets	#Ep	B27 (B*2705), mismatched eplets	#Ep	B61 (B*4002), mismatched eplets
1	A*0101	A*0201	B*1402	B*0702	Cw*0701	Cw*0702	7	11AMR, 44RTE, 76ERI, 82ALR, 113HN, 163L, 193PV	5	9H, 70AKA, 76ERT, 82LLR, 151RV	5	9H, 41T, 44RKE, 113HN, 151RV
2	A*0101	A*0201	B*0702	B*0801	Cw*0701	Cw*0702	6	44RTE, 76ERI, 82ALR, 131S, 163L, 193PV	5	9H, 70AKA, 76ERT, 82LLR, 131S	3	9H, 41T, 44RKE
3	A*0101	A*0201	B*0702	B*4501	Cw*0701	Cw*0702	5	44RTE, 76ERI, 82ALR, 113HN, 193PV	3	70AKA, 76ERT,82LLR	1	113HN
4	A*0101	A*2501	B*0702	B*0801	Cw*0701	Cw*0702	4	44RTE, 131S, 163L, 193PV	7	9H, 70AKA, 73TD, 76ERT, 82LLR, 113YH, 131S	3	9H, 41T, 44RKE
5	A*0101	A*0201	B*0702	B*4403	Cw*0501	Cw*0702	3	44RTE, 76ERI, 113HN	3	9H, 70AKA, 82LLR	2	9H, 113HN
6	A*0101	A*0201	B*4501	B*3901	Cw*0501	Cw*1701	3	44RTE, 76ERI, 82ALR	4	65QIA, 70AKA, 76ERT, 82LLR	0	none
7	A*0101	A*2501	B*5501	B*3701	Cw*0602	Cw*0702	2	116Y, 163L	3	70AKA, 113YH, 163E	4	41T, 44RKE, 116Y, 163E
8	A*0101	A*2501	B*3501	B*4101	Cw*0602	Cw*0401	0	None	8	44REE, 65QIA, 70AKA, 73TD, 76ERT, 82LLR, 113YH, 16	1	163E

different or shared between donor and patient. This analysis considers each donor HLA antigen mismatch towards the entire HLA-A, B, C phenotype of the recipient.

Table 1 shows examples with three mismatched antigens B51 (B*5101), B27 (B*2705) and B61 (B*4002) for eight different HLA-A, B, C phenotypes. Although they are all considered one-antigen mismatches by conventional matching criteria, they display marked differences in structural epitope compatibility. For certain HLA phenotypes a given mismatch has no or few mismatched epitopes (B51 for cases 7 and 8; B61 for cases 3, 5, 6 and 8) but for other phenotypes, the same HLA antigen has many mismatched epitopes (B51 for cases1, 2 and 3; B61 for case 5) and is therefore, structurally highly incompatible. Table 1 also illustrates that certain antigens such as B27 do not often have zero or few mismatched eplets. Altogether, the epitope load of a donor HLA mismatch is influenced by the recipient's HLA type representing a repertoire of self-epitopes to which no antibodies can be made.

The class II eplet version of HLAMatchmaker is based on 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 [8]. Conventional matching criteria for organ transplantation have always emphasized the importance of DR antigens. However, many studies have shown that the immunogenic products of other class II loci can elicit antibody responses that are detrimental to the allograft. Thus we must consider that each DR antigen mismatch (and often enough a match) will have an extra epitope load because of additional incompatibilities at the DRB3/4/5, DQ and DP loci. HLAMatchmaker can determine the extent of the class II epitope load. As an example, a patient who types as DR15, DR18 may have the following genotype: DRB1*1501, DRB5*0101, DQB1*0502, DQA1*0102/ DRB1*0302, DRB3*0101, DQB1*0402, DQA1*0401. Table 2 shows most common DR-DQ haplotypes of fourteen DR antigens and the numbers of corresponding mismatched eplets. Five antigens DR4, DR7, DR9, DR11 and DR12 have more than twenty mismatched eplets, a relatively high epitope load. Conversely, DR1, DR8 and DR16 have fewer than ten mismatched eplets. DR17 which is serologically similar to DR18 has no mismatched DRB eplets but there are nine mismatched DQB1 and eight DQA1 eplets. The 'self' DR15 antigen has a DQB1*0602 mismatch with six eplets. Although this example does not include HLA-DP, it illustrates how HLAMatchmaker can determine different epitope loads among the class II mismatches.

Effect of epitope load on the HLA antibody response

Early studies by Lobashevsky *et al.* [9] have shown that the number of immunogenic triplet mismatches offers a reliable predictive value for flow cytometry crossmatches with sera from highly sensitized renal patients. Dankers *et al.* [10•] demonstrated a strong correlation between the number of mismatched triplets and the incidence of

Table 2

Example of class II eplet load differences among serologically defined DR antigens

Patient									
DR15	DRB1*1501	DRB5*0101	DQB1*0502	DQA1*0102					
DR18	DRB1*0302	DRB3*0101	DQB1*0402	DQA1*0401					
DR antigen	DRB1	DRB3/4/5	DQB1	DQA1	Eplet total	DRB1eplets	DRB3/4/5 eplets	DQB1 eplets	DQA1 eplets
Donor									
DR1	DRB1*0101	None	DQB1*0501	DQA1*0101	9	5	0	2	2
DR4	DRB1*0401	DRB4*0101	DQB1*0301	DQA1*0302	42	8	14	9	11
DR7	DRB1*0701	DRB4*0101	DQB1*0202	DQA1*0201	41	10	14	10	7
DR8	DRB1*0801	None	DQB1*0402	DQA1*0401	4	4	0	0	0
DR9	DRB1*0901	DRB4*0101	DQB1*0303	DQA1*0302	36	6	14	5	11
DR10	DRB1*1001	None	DQB1*0501	DQA1*0101	12	8	0	2	2
DR11	DRB1*1101	DRB3*0202	DQB1*0301	DQA1*0501	22	3	2	9	8
DR12	DRB1*1201	DRB3*0202	DQB1*0301	DQA1*0501	26	7	2	9	8
DR13	DRB1*1301	DRB3*0101	DQB1*0603	DQA1*0103	12	2	0	7	3
DR14	DRB1*1401	DRB3*0202	DQB1*0503	DQA1*0104	11	4	2	2	3
DR15 (self)	DRB1*1501	DRB5*0101	DQB1*0602	DQA1*0102	6	0	0	6	0
DR16	DRB1*1601	DRB5*0202	DQB1*0502	DQA1*0102	2	0	2	0	0
DR17	DRB1*0301	DRB3*0101	DQB1*0201	DQA1*0501	17	0	0	9	8
DR18 (self)	DRB1*0302	DRB3*0101	DQB1*0402	DQA1*0401	0	0	0	0	0

humoral sensitization induced by a kidney transplant or developed during pregnancy. By contrast, there seems no significant association between triplet mismatching and cytotoxic T-cell precursor frequencies, an indicator of cellular alloimmune responsiveness [11]. Taylor's group at Cambridge University Hospitals in the United Kingdom have also reported a correlation between the number of mismatched triplets and eplets and the presence of HLA antibodies detected in Luminex assays with single class I alleles [12,13].

Epitope loads also affect anti-class II antibody responses. Donor-specific, DRB1-reactive antibodies are less often detectable than antibodies against other class II epitopes [14]. Antibody absence correlates with low numbers of mismatched DRB1 eplets. By contrast, donor-specific DRB3, 4 and 5 mismatches induce more antibody responses and they have higher numbers of incompatible eplets. Especially striking is the high incidence of antibodies against DRB4 (DR53) as also has been reported elsewhere [15]. This frequent antibody response is not really surprising because DR53 has a large array of unique eplets [8]. Antibodies against HLA-DQ are also more common and this correlates with more mismatched eplets on DQB and DQA than on DRB1 [14].

Altogether, these findings suggest that HLAMatchmaker has the potential of optimizing donor kidney allocation to reduce the problem of humoral alloimmunization.

Effect of HLA epitope load on transplant outcome

The triplet matching concept has clinical relevance as suggested by an analysis of the UNOS and Eurotransplant kidney transplant databases showing that HLA-A,B mismatched kidneys that are compatible at the triplet level exhibit almost identical graft survival rates as the zero HLA-A,B antigen mismatches defined by conventional criteria [16]. This beneficial effect of triplet matching applies to both non-sensitized and sensitized patients and also to white and non-white patients. Haririan *et al.* [17] have also shown that triplet matching can provide useful prognostic information about kidney transplantation in African-Americans. Very recently, Valentini *et al.* applied HLAMatchmaker to the selection of successful kidney transplants for two highly sensitized pediatric patients following desensitization treatment with intravenous immunoglobulin [18,19].

Although the group of Opelz concluded from their analysis of the Collaborative Transplant Database that triplet matching had no significant association with kidney graft survival [20], their data showed clearly similar five-year graft survivals for the zero-antigen mismatches and groups with zero or few triplet mismatches [21]. Interestingly, this group reported that mismatching for HLA-DP, especially at the level of structurally defined DPB epitopes has an adverse effect on kidney transplant survival [22]. 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 [23]. There is also an association between eplet numbers of donor HLA class I antigen mismatches and pediatric cardiac transplant rejection [24].

The epitope load concept applies also to platelet transfusions of refractory, thrombocytopenic patients who are

alloimmunized to HLA. Two studies have shown an association between successful transfusions and low numbers of HLAMatchmaker-determined triplets or eplets on donor platelets [25,26]. A recent report describes an HLAMatchmaker-based strategy for platelet transfusions in thrombocytopenic patients [27]. By contrast, triplet matching does not benefit patient survival after unrelated donor bone marrow transplantation although there is a slight effect on engraftment and acute graft-versus-host disease [28].

HLAMatchmaker-based analysis of serum antibody reactivity

HLAMatchmaker has primarily been used to analyze the reactivity patterns of sera from highly sensitized patients analysis and the identification of potential donors with acceptable mismatches. The group of Frans Claas at Leiden University Medical Center in The Netherlands has convincingly demonstrated that the primary purpose of serum screening must focus on the identification of acceptable mismatches for highly sensitized patients [29••,30•,31,32]. 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 [29••]. The application of HLAMatchmaker has enhanced the Acceptable Mismatch program [30•] and is now routinely used in Eurotransplant [32]. The cumulative frequencies of self-antigens and acceptable mismatches can be used to calculate the probability of finding a donor as an assessment of the transplantability of a sensitized patient [33].

Goodman *et al.* [12] have also used HLAMatchmaker in the analysis of sera from highly sensitized patients in single class I allele Luminex assays to determine acceptable mismatches. Other investigators have also demonstrated the usefulness of HLAMatchmaker to analyze antibody reactivity in different serum screening assays to identify suitable donors for retransplantation [34–38].

A recent study addressed the use of HLAMatchmaker to analyze the reactivity patterns of class II specific antibodies in transplant patients [14]. Sera from 75 sensitized patients were screened with single DR, DQ and DP heterodimers on a Luminex platform. About one-quarter of the patients had donor-specific antibodies induced by DRB1 eplets versus a 50% frequency of antibodies to DRB3 and DRB5 eplets and more than 80% frequency of antibodies to DRB4 eplets. Donor-specific antibodies to DQB eplets were found in more than 80% of the sera and anti-DQA eplet antibodies were detected with a frequency of about 60%. About one-third of the sera had anti-DP antibodies; they reacted predominantly with two DPB eplets and an allelic pair of DPA eplets.

The HLAMatchmaker analysis of serum screening results with single allele panels should consider high-resolution (four-digit) molecular typing of both antibody producer and immunizing donor to determine their exact immunogenetic relationship. Also, very helpful is the categorization of HLA sensitized patients according to the presence of sensitizing tissue: (1) sensitizing tissue is absent such as a previous transplant has been removed, or prior transfusion or pregnancy, (2) sensitizing tissue is present and may absorb donor-specific HLA antibodies especially class I and (3) the combination of both conditions.

The first step of an HLAMatchmaker analysis of serum antibody reactivity is to identify those alleles that give negative reactions. Such alleles can be expected to have eplets that are not recognized by patient's antibodies and from these eplets together with the patient's own eplets we can identify alleles that are acceptable mismatches. Certain antibodies react only with a pair of eplets on the same allele. This was first observed in a study on human monoclonal antibodies derived from a woman who had become HLA sensitized during pregnancy [39]. As an example, an antibody generated against 62QE of the immunizing HLA-A3 antigen reacted with only 62QE-carrying antigens except HLA-A30 and HLA-A31. All reactive antigens share with HLA-A3 a glycine residue in sequence position 56 whereas the non-reactive 62QE-carrying antigens have an arginine residue. Positions 56 and 62 are about 11 Å apart; this distance is sufficient to permit contact with two different complement determining region (CDR) loops of antibody. One is the antibody specificity-mediating CDR loop which interacts with 62QE and the other CDR loop interacts with 56G which as been referred to as a Critical Contact Site necessary for sufficient antibody binding. In other words, the epitope recognized by this monoclonal antibody is represented by a pair of eplets. Thus, another explanation for a negative reaction with antibody could be that the allele has the specific eplet but lacks the Critical Contact Site. In such case, acceptable mismatches would more difficult to identify. This concept may also explain why antibodies react with certain antigens in binding assays but not in complement-dependent lymphocytotoxicity [40].

HLAMatchmaker-based matching: avoid immunogenic epitopes

An epitope has two characteristics namely antigenicity, that is its reactivity with antibody, and immunogenicity, that is its ability of inducing an antibody response. Immunogenicity depends on the structural difference between an immunizing protein and the antibody responder's homologous proteins [41]. HLAMatchmaker can be used as a quantitative tool to determine the degree of a mismatch. As described above, the epitope load affects the HLA antibody response and allograft outcome. HLA-Matchmaker seems also useful as a qualitative tool to assess epitope immunogenicity determined by the frequency of a specific antibody response [42]. High

immunogenicity epitope mismatches should be avoided whereas low-immunogenicity epitopes might be considered permissible mismatches.

There is no structurally based prediction model for determining epitope immunogenicity. Possible factors include location and exposure of an epitope on the molecular surface, the relative difference in amino acid residue composition and perhaps the (HLA) genetic make up of the antibody responder. At present, a practical approach is to collect information about the frequencies of epitope-specific antibody responses in context with the exposure rate to epitope mismatches [43]. One possibility is to analyze serum screenings on patients with rejected kidney transplants who have undergone allograft nephrectomy. HLAMatchmaker-based analysis reveals restricted antibody specificity patterns against certain structurally defined epitopes on immunizing donor HLA antigens whereas other donor epitopes do not react [44•]. Donor specific antibody reactivity may persist for long periods of time after allograft nephrectomy but often enough it diminishes and even become undetectable after a few months.

A preliminary study conducted under auspices of the 14th International HLA Workshop, has shown differences between class I eplet immunogenicity [43]. Serum screening was limited to lymphocytotoxicity assays and in some instances, antigen-binding assays such as Elisa and Flow beads. Almost no data were available about antibody responses to class II epitopes. This study is continuing as a 15th International HLA Workshop project. All serum screenings include Luminex assays with single HLA-A, B, C and HLA-DR, DQ and DP alleles so that HLA antibody reactivity patterns can be analyzed in more precise detail. We expect that informative allograft nephrectomy cases will generate reasonable estimates about epitope immunogenicity following kidney transplantation. Such information will be useful for the development of a donor-selection strategy based on permissible mismatching.

Conclusions

HLAMatchmaker is clinically useful in the management of transplant patients and in providing platelet transfusion support of thrombocytopenic patients. It permits a structural assessment of donor–recipient compatibility and the identification of antibodies against epitopes on HLA class I and class II alleles can determine mismatch acceptability for sensitized patients.

HLAMatchmaker programs, publications and tutorials can be downloaded free of charge from the website: http://www.HLAMatchmaker.net.

Acknowledgement

The HLAMatchmaker studies by the author are supported by Grant RO1-AI-55933 from the National Institutes of Health.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cai J, Terasaki PI, Mao Q, Pham T, El-Awar N, Lee JH, Rebellato L, 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**:2947-2954.

2. El-Awar N, Lee JH, Tarsitani C, Terasaki PI, El-Awar N, Lee J-H, Tarsitani C, Terasaki PI: **HLA class I epitopes: recognition of binding sites by mAbs or eluted alloantibody confirmed with single recombinant antigens**. *Hum Immunol* 2007, **68**:170-180.

3. El-Awar NR, Akaza T, Terasaki PI, Nguyen A, El-Awar NR, Akaza T, Terasaki PI, Nguyen A: **Human leukocyte antigen class I epitopes: update to 103 total epitopes, including the C locus**. *Transplantation* 2007, **84**:532-540.

4. Terasaki P: **Post transplantation monitoring and HLA antibody epitope identification**. *Curr Opin Immunol* 2008, **(20)**: in press.

5. Chen J, Anderson JB, DeWeese-Scott C, Fedorova ND, Geer LY, He S, Hurwitz DI, Jackson JD, Jacobs AR, Lanczycki CJ *et al.*: **MMDB: Entrez's 3D-structure database**. *Nucleic Acids Res* 2003, **31**:474-477.

6. • Duquesnoy RJ: **HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. I. Description of the algorithm**. *Hum Immunol* 2002, **63**:339-352.
This is the original description of the triplet version of HLAMatchmaker.

7. •• Duquesnoy R: **A structurally based approach to determine HLA compatibility at the humoral immune level**. *Hum Immunol* 2006, **67**:847-862.
This paper describes the rationale for the eplet version of HLAMatchmaker based on concepts emerging from reviewing the stereochemical structure of various protein antigen–antibody complexes and the role of critical amino acids in these interactions.

8. 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.

9. Lobashevsky AL, Senkbeil RW, Shoaf JL, Stephenson AK, Skelton SB, Burke RM, Deierhoi MH, Thomas JM: **The number of amino acid residues mismatches correlates with flow cytometry crossmatching results in high PRA renal patients**. *Hum Immunol* 2002, **63**:364-374.

10. • Dankers MKA, Witvliet MD, Roelen DL, De Lange P, Korfage N, Persijn GG, Duquesnoy RJ, Doxiadis IIN, Claas FHJ: **The number of amino acid triplet differences between patient and donor is predictive for the antibody reactivity against mismatched HLA antigens**. *Transplantation* 2004, **I28**:.
This paper describes strong correlations between the triplet load of mismatched class I antigens and the HLA antibody response after kidney transplantation and during pregnancy.

11. Dankers MKA, Heemskerk MHM, Duquesnoy RJ, Doxiadis I, Oudshoorn M, Roelen DL, Claas FHJ: **HLAMatchmaker algorithm is not a suitable tool predict the alloreactive cytotoxic T lymphocyte reponse *in vitro***. *Transplantation* 2004, **78**:165-171.

12. 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**:1331-1336.

13. Kosmoliaptsis V, Bradley J, Sharples L, Chaudhry A, Key T, Goodman R, Taylor C: **Predicting the immunogenicity of HLA class I alloantigens using structural epitope analysis determined by HLAMatchmaker**. *Transplantation* 2008, **85**:1817-1825.

14. Duquesnoy R, Awadalla Y, Lomago J, Jelinek L, Howe J, Zern D, Hunter B, Martell J, Girnita A, Zeevi A: **Retransplant candidates have donor-specific antibodies that react with structurally**

defined HLA-DR, DQ, DP epitopes. *Transpl Immunol* 2008, **18**:352-360.

15. Worthington JE, Martin S, Al-Husseini DM, Dyer PA, Johnson RW: **Posttransplantation production of donor HLA-specific antibodies as a predictor of renal transplant outcome**. *Transplantation* 2003, **75**:1034-1040.

16. 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**:884-889.

17. Haririan A, Fagoaga O, Daneshvar H, Morawski K, Sillix D, El-Amm J, West M, Garnick J, Migdal S, Gruber S *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**:226-233.

18. Valentini RP, Nehlsen-Cannarella SL, Gruber SA, Mattoo TK, West MS, Lang C, Imam AA: **Intravenous immunoglobulin, HLA allele typing and HLAMatchmaker facilitate successful transplantation in highly sensitized pediatric renal allograft recipients**. *Pediatr Transplant* 2007, **11**:77-81.

19. Duquesnoy RJ: **Match and Treat', an effective strategy for transplanting highly sensitized pediatric transplant candidates? (Editorial)**. *Pediatr Transplant* 2007, **11**:3-4.

20. Laux G, Mytilineos J, Opelz G: **Critical evaluation of the amino acid triplet-epitope matching concept in cadaver kidney transplanation**. *Transplantation* 2004, **77**:902-907.

21. Duquesnoy R, Claas F: **Is the application of HLAMatchmaker relevant in kidney transplantation? (Letter to the Editor)**. *Transplantation* 2005, **79**:250-251.

22. Laux G, Mansmann U, Deufel A, Opelz G: **A new epitope-based HLA-DP matching approach for cadaveric kidney transplantation**. *Transplantation* 2003, **75**:1527-1532.

23. Boehringer D, Reinhard T, Duquesnoy R, Boehringer S, Enczmann J, de Lange P, Claas F, Sundmacher R: **Beneficial effect of matching at the HLA-A and B amino-acid triplet level on rejection free survival in penetrating keratoplasty**. *Transplantation* 2004, **77**:417-421.

24. Perasaari J, Viskari J, Jalanko H, Merenmies J: **Eplet mismatches determined by HLAMatchmaker associate with anti-HLA antibodies, rejections and coronary artery disease after paediatric heart transplantation**. *Tissue Antigens* 2008, **71**:291 (Abstract).

25. Nambiar A, Duquesnoy RJ, Adams S, Oblitas J, Leitman S, Stroncek D, Marincola F: **HLAMatchmaker-driven analysis of response to HLA matched platelet transfusions in alloimmunized patients**. *Blood* 2006, **107**:1680-1687.

26. Brooks E, MacPherson B, Fung M: **Validation of HLAMatchmaker algorithm in identifying acceptable HLA mismatches for thrombocytopenic patients refractory to platelet transfusions**. *Transfusion* 2008, in press.

27. Duquesnoy R: **Structural epitope matching for HLA alloimmunized thrombocytopenic patients: a new strategy to provide more effective platelet transfusion support?** *Transfusion* 2008, **I48**:221-227.

28. Duquesnoy R, 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, in press.

29. •• 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**:190-193.
This paper describes the application of HLAMatchmaker in the acceptable mismatch program for successful kidney transplantation of highly sensitized patients.

30. • 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**:31-38.
This paper describes an HLAMatchmaker-based analysis of sera from highly sensitized patients and its role in the Acceptable mismatch program.

31. Claas FHJ, Dankers MK, Oudshoorn M, van Rood JJ, Mulder A, Roelen DL, Duquesnoy RJ, Doxiadis IIN: **Differential immunogenicity of HLA mismatches in clinical transplantation**. *Transpl Immunol* 2005, **14**:187-191.

32. Doxiadis IIN, Duquesnoy RJ, Claas FHJ: **Extending options for highly sensitized patients to receive a suitable kidney graft**. *Curr Opin Immunol* 2005, **17**:536-540.

33. 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**:889-897.

34. 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**:1728-1731.

35. Varnavidou-Nicolaidou A, Doxiadis I, Iniotaki-Theodoraki A, Patargias T, Stavropoulos-Giokas C, Kyriakides G: **HLA class I donor-specific triplet antibodies detected after renal transplantation**. *Transplant Proc* 2004, **36**:1732-1734.

36. Rosenberg J, Berrri M, Jackowski M, Levis D, Nehlsen-Cannarella S, Oh H: **Multi-array antibody screening in detecting antibodies to mismatched HLA in patients awaiting a second transplant**. *Transplant Proc* 2006, **38**:3393-3395.

37. Mihaylova A, Baltadjieva D, Boneva P, Ivanova M, Penkova K, Marinova D, Mihailova S, Paskalev E, Simeonov P, Naumova E: **Clinical relevance of anti-HLA antibodies detected by flow-cytometry bead-based assays—single-center experience**. *Hum Immunol* 2006, **67**:787-794.

38. Glehn-Ponsirenas R, Paladino-Vieira B, Schneider R, Contini-Duarte D, Glehn C: **PRA and HLAMatchmaker rerults and their influence in deceased organ donor distribution: how to determine the best recipient for deceased organs**. *Tissue Antigens* 2008, **71**:289 [Abstract].

39. Duquesnoy RJ, Mulder A, Askar M, Fernandez-Vina M, Claas FHJ: **HLAMatchmaker-based analysis of human monoclonal antibody reactivity demonstrates the importance of an additional contact site for specific recognition of triplet-defined epitopes**. *Hum Immunol* 2005, **66**:749-761.

40. Duquesnoy RJ: **HLAMatchmaker: a molecularly based algorithm for histocompatibility determination**. *ASHI Q* 2002:60-62.

41. Benjamin DC, Berzofsky JA, East IJ, Gurd FRN, Hannum C, Leach SJ, Margoliash E, Michael JG, Miller A, Prager EM *et al.*: **The antigenic structure of proteins: a reappraisal**. *Ann Rev Immunol* 1984, **2**:67-101.

42. Duquesnoy RJ, Marrari M: **HLAMatchmaker: a molecularly based algorithm for histocompatibility determination. II. Verification of the algorithm and determination of the relative immunogenicity of amino acid triplet-defined epitopes**. *Hum Immunol* 2002, **63**:353-363.

43. Duquesnoy RJ, Claas FHJ: **Progress report of 14th international histocompatibility workshop project on the structural basis of HLA compatibility**. *Tissue Antigens* 2007, **69(Suppl. 1)**:180-184.

44. • Adeyi OE, Girnita A, Awadalla Y, Askar M, Shapiro R, Howe J, Martell J, Zeevi A, Nalesnik M, Rhandawa P *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.
This report shows that donor-triplet reactive antibodies become readily detectable in sera following removal of a rejected kidney transplant.