ORIGINAL ARTICLES

HLA MATCHMAKER: A MOLECULARLY BASED ALGORITHM FOR HISTOCOMPATIBILITY DETERMINATION

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INTRODUCTION

In organ transplantation, donor-recipient histocompatibility is generally determined by counting the number of mismatched antigens in the donor's HLA-A, B, DR phenotype. Many studies have shown that zero-HLA antigen mismatched transplants have the highest success rates and these findings have led to the allocation policy of mandatory sharing of zero-mismatched cadaver kidneys. Another method for assessing donor-recipient compatibility considers public epitopes that are shared by HLA antigens belonging to so-called cross-reacting antigen groups (CREGs). Several studies have suggested CREG matching is associated with graft survivals that are better or comparable to those allocated on HLA antigen matching, but others have not found a beneficial effect of CREG matching. The controversy about CREG matching is due in part to the difficulty to define the exact spectrum of public epitopes on all HLA antigens. The CREG matching system in UNOS utilizes ten CREGs but the actual number of public epitopes is considerably higher. Amino acid sequence information on HLA antigens has led to the identification of polymorphic residues that correspond to many public epitopes and private HLA antigens.

HLA matching is important for alloimmunized patients and serum screening may yield information about specific antibodies to public epitopes and the HLA antigens in the corresponding CREGs are considered unacceptable mismatches. Serum reactivity patterns are usually analyzed by 2x2 table statistics but this correlation analysis does not permit a meaningful interpretation if the Panel Reactive Antibody (PRA) activity exceeds 85%. Often enough, many highly sensitized patients remain on the waiting list with little prospect of a transplant because the probability of finding a zero antigen mismatch is very low.

HLA Matchmaker is a computer algorithm especially designed to identify compatible HLA antigens for highly allosensitized patients [1-3]. Donor-recipient HLA compatibility is assessed at the structural level by intralocus and interlocus comparisons of polymorphic amino acid triplet sequences in alloantibody-accessible positions of HLA molecules namely, the α-helices and β-loops of the protein chain structure. The residues in the strands of the β-pleated sheets of the peptide-binding groove are excluded from this matching algorithm because they cannot make direct contact with alloantibodies.

Each triplet is designated by its amino acid composition around a given position in the amino acid sequence. Amino acid residues are marked with the standard letter code; an uppercase letter corresponds to the residue in the numbered position whereas the lowercase letters describe the nearest neighboring residues. For instance, the triplet a65rNm represents an asparagine residue (N) in position 65 with arginine (r) in position 64 and methionine (m) in position 66 of the HLA-A chain. Many triplets are marked with one or two residues

because their neighboring residues are the same on all HLA Class I chains and they are therefore not shown. For instance, b12aM represents an alanine residue in position 11 and a methionine residue in position 12 on HLA-B chains. The triplet b41T has a threonine in position 41 and the two neighboring monomorphic residues are not shown.

HLA Matching at the Triplet Level

HLAMatchmaker applies two principles: (1) each HLA antigen represents a distinct string of polymorphic triplets as potential immunogens that can induce specific alloantibodies and, (2) patients cannot make alloantibodies against triplets on their own HLA molecules [2]. The algorithm assesses donor-recipient compatibility through intralocus and interlocus comparisons, and determines what triplets on mismatched HLA molecules are 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.

As an example let us consider a kidney donor who types as HLA-A2,A32; B8,B55; Cw3,Cw6 There are two transplant candidates: patient A types as HLA-A2,A30; B18,B27; Cw2,Cw4 and patient B types as HLA-A2,A31; B42,B53; Cw2,Cw7. This donor is a three-HLA-A, B antigen mismatch for both patients. Which patient is the best recipient? HLAMatchmaker determines if triplets on a donor antigen are found in the same sequence position on any of the patient's HLA antigens. For instance, the donor's HLA-B8 represents is a mismatched antigen by conventional criteria but at the structural level, HLA-B8 has six mismatched triplets for the HLA-A2,A30; B18,B27; Cw2,Cw4 but has no mismatched triplets for HLA-A2,A31; B42,B53; Cw2, Cw7 (Table 1). Altogether, this donor's HLA antigens have a total of 14 different mismatched triplets for patient A (A32: 3 triplets, B8: 6 triplets, B55: 3 triplets, Cw3: 2 triplets and Cw6: 1 triplet), but for patient B, all of them are zero-triplet mismatches. Thus, at the structural level, this donor appears fully compatible to patient B but not to patient A. This matching algorithm is at the humoral immune level and is particularly useful for highly alloimmunized patients.

Application of HLAMatchmaker after Serum Analysis of Highly Sensitized Patients

The number of HLA antigens with zero or few triplet mismatches depends on the HLA phenotype of the patient. For some patients is it difficult to find HLA antigens matched at the triplet level. As an example, patient 3 typed as HLA-A3,A11; B18,B62, Cw7,- and was highly sensitized with a PRA of 96%. HLAMatchmaker analysis showed B75 as a zero-triplet mismatch and B72 and B76 as one-triplet mismatches. These antigens have very low frequencies in our donor population and it appeared unlikely that HLAMatchmaker would increase compatible donor availability for this patient. Our PRA analysis showed that 48 of 50 HLA-typed panel cells reacted with patient's serum. Two panel cells gave consistently negative reactions. From their phenotypes, HLA-A3,A26; B62,- and HLA-A11,-; B18,B51, it should be noted that these negative cells shared HLA antigens with the patient. These negative cells expressed two mismatched antigens: A26 and B51. Table 2 shows that A26 has eight mismatched triplets and B51 has five mismatched triplets. Because patient's antibodies did not react with A26 or B51, these triplets were considered acceptable mismatches. After entering A26 and B51 as "negative antigens" in HLAMatchmaker we identified eight additional HLA antigens that were zero/acceptable triplet mismatches namely, A25, A32, A66, A74, B35, B52, B53 and B77. This example illustrates how a serum analysis combined with HLAMatchmaker can be useful in strategies to increase donor availability for highly sensitized patients.

Triplet matching and Kidney Transplant Survival

Recent studies have been conducted to determine how class I HLA matching at the triplet level affects kidney transplant outcome. We have analyzed two multi-center databases of zero-HLA-DR mismatched kidneys transplanted in 1987-1999. One dealt with 31879 primary allografts registered by US transplant centers in the United Network for Organ Sharing (UNOS) database and the other consisted of 15872 transplants in the Eurotransplant program.

The results show that HLA-A,B mismatched

Table 1. Examples of HLA compatibility determination at the amino acid triplet level.

Patient 1	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	138
A2	F	sV	R	gR	A	Me	G	Ge	rKv	aHs	H	Vd	gTl	lRg	A	S	W	K	R	T
A30	S	sV	R	gS	A	Me	R	Qe	rNv	aQs	D	Vd	gTl	lRg	A	S	G	N	R	T
B18	H	sV	R	GR	A	Te	G	Rn	qIs	tNt	Y	Es	rNl	lRg	A	P	G	N	s	T
B27	H	sV	R	gR	A	Ee	G	Re	qIc	aKa	D	Ed	rTl	lLr	A	P	G	N	S	T
Cw2	Y	aV	R	sR	A	Ge	G	Re	qKy	rQa	D	Vn	rNl	lRg	A	P	G	N	R	T
Cw4	S	sV	W	gR	A	Ge	G	Re	qKy	rQa	aD	Vn	rNl	lRg	D	P	G	N	R	T
Donor HLA-B8	D	aM	R	gR	A	Ee	G	Rn	qIf	tNt	D	Es	rNl	lRg	A	P	G	N	R	T

Patient 1(contd)	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
A2	T	tKh	W	aAh	aHv	L	A	T	Ew	Y	Et	Q	A	K	Av	A	S	A	V	Q
A30	I	tQr	W	aAr	aRw	L	A	T	Ew	Y	Et	Q	P	K	Pi	A	G	A	V	E
B18	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	P	K	Pi	A	G	A	V	E
B27	I	tQr	W	aAr	aRv	L	A	E*	Ew	Y	Et	Q	P	K	Pi	A	G	A	V	E
Cw2	I	tQr	W	aAr	aRe	W	A	E*	Ew	Y	Et	Q	Eh	K	Pv	A	G	A	V	E
Cw4	I	tQr	W	aAr	aRe	R	A	T	Ew	Y	Et	Q	Eh	K	Pv	A	G	A	V	E
Donor HLA-B8	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	P	K	Pi	A	G	A	V	E

Patient 2	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	138
A2	F	sV	R	gR	A	Me	G	Ge	rKv	aHs	H	Vd	gTL	lRg	A	S	W	K	R	T
A31	T	sV	R	gR	A	Me	R	Qe	rNv	aHs	iD	Vd	gTL	lRg	A	S	G	N	R	T
B42	Y	sV	R	gR	A	Ee	G	Rn	qIy	aQa	D	Es	rNl	lRg	A	P	G	N	R	M
B53	Y	aM	R	gR	A	Te	G	Rn	qIf	tNt	Y	En	rIa	aLr	A	P	G	N	S	M
Cw2	Y	aV	R	sR	A	Ge	G	Re	qKy	rQa	D	Vn	rKl	lRg	A	P	G	N	R	T
Cw7	D	aV	R	gR	A	Ge	G	Re	qNy	rQa	aD	Vs	rNl	lRg	D	P	G	N	R	T
Donor HLA-B8	D	aM	R	gR	A	Ee	G	Rn	qIf	tNt	D	Es	rNl	lRg	A	P	G	N	R	M

Patient 2 (contd)	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
A2	T	tKh	W	aAh	aHv	L	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	A	V	Q
A31	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	dP	K	Av	A	S	S	V	Q
B42	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	dP	K	Pi	A	G	A	V	E
B53	I	tQr	W	aAr	aRv	L	A	L	Ew	Y	Et	Q	dP	K	Pv	A	G	A	V	E
Cw2	I	tQr	W	aAr	aRe	W	A	E	Ew	Y	Et	Q	eH	K	Pv	A	G	A	V	E
Cw7	I	tQr	L	aAr	aRa	L	A	T	Ew	Y	Et	Q	eP	K	Pl	A	G	A	V	Eq
Donor HLA-B8	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	dP	K	Pi	A	G	A	V	E

Analysis was based on the following molecular types. Patient 1: HLA-A*0201, A*3001; B*1801,B*2705; C*0202,C*0401. Patient 2: HLA-A*0201,A*3101; B*4201,B*5301; C*0202, C*0701. Donor: HLA-A*0201,A*3201; B*0801,B*5501; C*0302,C*0602

kidneys that were compatible at the triplet levelexhibited almost identical graft survival rates as the zero HLA-A,B antigen mismatches defined by conventional criteria [4]. This beneficial effect of triplet matching was seen for both non-sensitized and sensitized patients and also for white and non-white patients. The practical implication of this finding is the possible use of the HLAMatchmaker algorithm to increase the number of well-matched transplants [5]. Furthermore, triplet matching may benefit especially sensitized patients and non-white transplant candidates for whom it is difficult to find donors with good matches.

Why do the zero-triplet and few-triplet mismatched transplants appear so successful? Designed originally for highly sensitized patients [1, 2], the HLAMatchmaker algorithm considers only triplets that are in antibody-accessible positions of the HLA molecular structure. HLA-specific antibodies play a major role in graft rejection [6] and matching at the humoral immune level, i.e. for epitopes recognizedby such antibodies can be expected to improve graft

Table 2. Identification of acceptable triplet mismatches from negatively reacting panel cells mismatched for HLA-A26 and HLA-B51.

Patient 3	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	138
A3	F	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	lRg	A	S	G	N	R	T
A11	Y	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	lRg	D	P	G	N	R	T
B18	H	sV	R	gR	A	Te	G	Rn	qIs	tNt	Y	Es	rNl	lRg	A	P	G	N	S	M
B62	Y	aM	R	gR	A	Ma	G	Re	qIs	tNt	Y	Es	rNl	lRg	A	P	G	N	S	M
A26 Mismatch	Y	sV	R	gR	A	Me	G	Rn	rNv	aHs	D	An	gTL	lRg	D	P	G	N	R	T
B51 Mismatch	Y	aM	R	gR	A	Te	G	Rn	qIf	tNt	Y	En	rla	aLr	A	P	G	N	S	M

Patient 3 (contd)	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253
A03	I	tKr	W	aAh	aHe	L	A	dT	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
A11	I	tKr	W	aAh	aHa	Q	A	R	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
B18	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	E
B62	I	tQr	W	aAr	aRe	W	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	E
A26 Mismatch	I	tQr	W	tAh	aHe	W	A	R	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Q
B51 Mismatch	I	tQr	W	aAr	aRe	L	A	L	Ew	H	Et	Q	dP	K	Pv	A	G	A	V	E

Analysis was based on the following molecular types: Patient 3: HLA-A*0301,A*1101; B*1801,B*1501. Mismatched antigens: HLA-A*2601 and HLA-B*5101.

survival. HLA compatibility must also consider cellular immune mechanisms of graft rejection such as class I HLA specific cytotoxic T-lymphocytes and indirect allorecognition of processed donor class I HLA antigens presented by recipient CD4 T-cells. Although HLA matching at the cellular immune level must use different structural criteria (they are currently not defined), this humoral immunity-based matching strategy permits the identification of triplet matches among the zero-HLA-DR mismatches.

Why does HLA Matchmaker use Triplets?

The question can be answered by examining how antigens react with specific alloantibodies. Three-dimensional structures of different antigen-antibody complexes have shown that up to six hypervariable loops (or Complementarity Determining Regions, CDRs) of the antibody binding sites make contact with protein antigen. The contact area between antibody and antigen is about 700 to 800 square Angstroms and this is similar to the size of the HLA molecular surface seen from above the peptide-binding region and the alpha helices. . An epitope on a protein antigen contains a few critical residues that provide dominant contributions to the binding energy with one of the CDRs. There are also many contact residues that interact with other CDRs to increase the overall association of the immune complex, but they are not necessarily required for antibody specificity. Thus, in the case of an HLA-specific antibody, it seems likely that one CDR plays a primary role in the specific binding with a polymorphic triplet whereas the other CDRs interact with other sites on the HLA molecule; such sites may have monomorphic and/or polymorphic residues.

This concept may increase our understanding of the reactivity of complement-dependent, lymphocytotoxic alloantibodies against HLA and why their detection is so often technique-dependent. As an example, let us examine the reactivity of antibodies against A25 and A26. More than twenty-five years ago, we published two articles on what was considered that time, two new splits of HLA-A10 [7, 8]. Our serum screening efforts had identified several monospecific anti-A25 and anti-A26 sera that were used for typing purposes by many laboratories worldwide. Absorption/elution studies showed that these sera exhibited CYNAP (i.e. the Cytotoxicity-Negative, Adsorption-Positive phenomenon) towards the other split of A10 whereas anti-A10 antibodies reacted with both A25 and A26 cells by complement-dependent lymphocytotoxicity. How are these findings explained?

Since we had HLA typing information about antibody producers and immunizers, it was possible to conduct an HLAMatchmaker analysis to determine what triplets were different on the immunizing HLA antigen (Table 3). In all cases, the antibodies appeared specific for 150tAh and this triplet is

Table 3. Triplet matching analysis of the serological reactivity of antibodies induced by HLA-A25 and HLA-A26

Serum	HLA-type Ab Producer	Ag	Reaction	Unshared Triplets on Antigen
Jun (anti-A25)	A1,A3;B7,B8	A25*	CYT+	80rIa 82aLr 150tAh 156W 183A 193Av
		A26	CYNAP	150tAh 156W 183A 193Av
Schu (anti-A25)	A2,A68;B37,-	A25*	CYT+	76Es 80rIa 82aLr 90D 150tAh 163R
		A26	CYNAP	76An 90D 150tAh 163R
Mich (anti-A26)	A3,A23; B7,B13	A26*	CYT+	76An 90D 150tAh 156W 163R 183A 193Av
		A25	CYNAP	90D 150tAh 156W 163R 183A 193Av
Tyl (anti-A26)	A2,A11; B13,B62	A26*	CYT+	62Rn 76An 150tAh
		A25	CYNAP	62Rn 80rIa 82aLr 150tAh
Sand (anti-A10)	A1,A24; B7,B44	25*	CYT+	66rNv 150tAh 156W 183A 193Av
		A26	CYT+	66rNv 150tAh 156W 183A 193Av
Elli (anti-A10)	A1,A32; B13, B14	A26*	CYT+	150tAh 156W
		A25	CYT+	150tAh 156W

* Immunizing HLA antigen

found exclusively on HLA-A10 molecules, including A25 and A26. In the case of the A25-specific typing sera Jun and Schu, the antibody producer had been exposed to 150tAh and five other triplets on the immunizing A25 antigen. This antibody showed lymphocytoxicity reactivity towards A25 but CYNAP activity towards A26. It seems likely that one CDR of this antibody reacted with 150tAh but this reaction by itself was not strong enough for the antibody to bind C1q, the first component of complement necessary for initiating the process leading to lymphocytotoxicity. Apparently, a second CDR of this antibody must react with another part of the antigen so that the overall binding strength becomes sufficient for C1q activation. The unshared triplets that are present on A25 but not A26 appear to be the most likely recognition sites for the second CDR. Underlined in Table 1, they are 80rIa and 82aLr for antibody Jun and 76Es, 80rIa and 82aLr for antibody Schu. It should be noted that the Jun and Schu antibodies do not see these triplets as specific epitopes because they do not react with 150tAh-negative cells that express 76Es, 80rIa or 82aLr.

Similar explanations can be offered for the reactivity of Mich and Tyl antibodies that exhibit lymphocytotoxicity towards A26 but CYNAP towards A25. In this case, the 76An triplet seems necessary for complement-dependent lymphocytotoxicity (Table 3). This analysis included also two sera that were specific for A10, i.e. A25+A26. The Sand antibodies were induced by A25 and the Elli

antibodies were induced by A26. These antibodies showed lymphocytotoxicity against both A25 and A26 and HLAMatchmaker analysis showed no differences in unshared triplets between A25 and A26. Apparently, the second CDR needed for C1q binding and complement activation must have reacted with a triplet shared between A25 and A26.

In conclusion, these findings may provide some insight about the reactivity of alloantibodies with HLA antigens. One triplet presented by the immunizing antigen reacts with one CDR that apparently conveys the specificity of the antibody. Such antibody can be expected to react other antigens that express the same triplet, in other words a cross-reacting group of antigens. The remaining CDRs of the antibody must interact with other triplets originally present on the immunizing HLA molecule to increase the energy release during the formation of the antigen-antibody complex so that C1q binding and subsequent complement activation and lymphocytotoxicity can take place. Cross-reacting antigens must have such triplets if they give positive reactions in direct complement-dependent lymphocytotoxicity. Absence of such triplets will diminish the binding energy release between antibody and the cross-reacting antigen as manifested by a negative lymphocytoxicity reaction although binding such as by ELISA testing might still be seen. The application of anti-human globulin (AHG) would be a useful source of additional binding energy for the activation of the complement cascade leading to lymphocytotoxicity.

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