

# 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

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**ABSTRACT:** HLAMatchmaker is a computer algorithm that assesses human leukocyte antigen (HLA) compatibility at the structural level by intralocus and interlocus comparisons of polymorphic amino acid triplets in antibody-accessible sequences of HLA class I molecules. This program permits the identification of mismatched HLA antigens that share all of their polymorphic triplets with the patient's HLA antigens and, therefore, could be considered fully compatible. The validity of this algorithm has been verified by analyzing the antibody specificity patterns of 127 well-characterized sera (panel reactive antibody [PRA] > 80%) that had been screened by direct complement-dependent and/or anti-human globulin augmented lymphocytotoxicity testing with large HLA-typed cell panels. A 2×2 table-based Chi-square analysis program was applied to determine positive and negative correlations between serum reactivity and the presence

HLA triplets assigned from the HLA types in the cell panel. The results indicate that high PRA patients do not produce antibodies to shared triplets on mismatched HLA antigens. Moreover, this serum analysis has permitted the identification of triplets with different degrees of immunogenicity as indicated by the frequencies of positive and negative correlations of serum reactivity with the HLA-typed cell panel. Mismatching for triplets with low immunogenicity provides further opportunities for identifying donors with acceptable HLA mismatches for highly sensitized patients. *Human Immunology* 63, 353–363 (2002). © American Society for Histocompatibility and Immunogenetics, 2002. Published by Elsevier Science Inc.

**KEYWORDS:** HLAMatchmaker; triplet; histocompatibility; immunogenicity

## INTRODUCTION

HLAMatchmaker is a computer algorithm designed to identify compatible human leukocyte antigens (HLA) for highly allosensitized patients without the need for extensive serum screening [1,2]. 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. This algorithm applies two

concepts: (1) each HLA antigen represents a string of multiple triplet-defined epitopes that have the potential of inducing humoral immune responses, and (2) allosensitized patients cannot produce antibodies to triplets on mismatched HLA antigens if such triplets are present in the same sequence location of any of the patient's own HLA molecules. HLAMatchmaker can identify HLA antigens that share all their triplets with the HLA antigens of the patient and, therefore, must be considered fully compatible at the epitope level. HLAMatchmaker permits the identification of additional HLA antigen mismatches that are acceptable as determined from the triplet information on HLA-typed panel cells that do not react with patient's serum [1,2].

This report describes serologic studies designed to verify the validity of the HLAMatchmaker algorithm.

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We have analyzed the antibody reactivity patterns of patient sera with high panel reactive antibody (PRA) activity determined by screening with large, HLA class I typed cell panels using lymphocytotoxicity-based methods. This analysis addressed the premise of HLAMatchmaker that allosensitized patients do not produce antibodies against polymorphic triplets on mismatched HLA antigens if such triplets are shared with the patient's own HLA antigens. Triplet sharing can be between different HLA antigens encoded by the same locus (intralocus matches) and between HLA antigens encoded by different loci (interlocus matches). The latter include triplets that are polymorphic at one locus but monomorphic at another locus.

Serum reactivity patterns of 127 high PRA sera were analyzed for their correlations with HLA antigens and triplet markers on panel cells using  $2 \times 2$  table chi-square statistical methods. This analysis yielded information about the frequencies of positive correlations (possible presence of antibody) and negative correlations (absence of antibody) for each triplet. The data are consistent with the concept that shared triplets do not induce specific alloantibodies. This analysis has also provided information about the relative immunogenicity of mismatched triplets. Certain triplets exhibit high immunogenicity whereas others have intermediate or low immunogenicity. Mismatching for triplet-associated epitopes with low immunogenicity provides further opportunities for identifying donors with acceptable HLA mismatches.

## MATERIALS AND METHODS

### Sources of High PRA Sera

The validity of HLAMatchmaker was investigated with a comprehensive analysis of the panel reactivity patterns of two groups of sera from highly sensitized patients with PRA values  $>80\%$ . One group consisted of 62 high PRA sera screened through a multilaboratory study conducted under auspices of the 12<sup>th</sup> International Histocompatibility Workshop [3, 4]. Serum screening was done by the complement-dependent, direct (CDC) and anti-human globulin (AHG) augmented lymphocytotoxicity techniques under standardized testing. After the application of several quality-control procedures [3], a final data set with a 534-cell panel tested in 14 laboratories was selected for antibody-specificity analysis. The median PRA value of these 62 sera was 91% (range: 80%–98%) by AHG screening and 84% (range: 62%–97%) by CDC screening.

The second group consisted of 65 high PRA sera from tissue-typing laboratories in Region 2 of the United Network of Organ Sharing (UNOS). The sera had been screened by CDC methods in multiple laboratories

against 200–500 HLA-typed cell panels and their median PRA value was 86% (range 80%–96%).

### Statistical Analysis of Serum Reactivity With Cell Panel

Serum reactivity analysis was done by  $2 \times 2$  table chi-square statistics with a previously reported Multiscreen program [5]. This computer program generates data about statistical correlations between serum reactivity patterns and the presence of HLA antigenic markers in the cell panel. These markers included the serologically defined HLA-A and HLA-B antigens and their polymorphic triplets assigned from sequence information of HLA antigens illustrated in Figure 1. As described before [1], each triplet is designated by its amino acid composition around a given position in the molecular sequence. Amino acid residues are marked with the letter code; an uppercase letter corresponds to the residue in the numbered sequence position, whereas the lowercase letters describe the nearest neighboring residues. For instance, the triplet a70aQs represents a glutamine residue (Q) in position 70 with alanine (a) in position 69 and serine (s) in position 71 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, therefore, they are not shown. For instance, ab62Ge represents a glycine (G) residue in position 62 and glutamic acid (e) residue in position 63 on HLA-A and HLA-B chains. The triplet a127K has a lysine in position 127 of HLA-A chains and the two neighboring monomorphic residues are not shown.

The Multiscreen program determines positive and negative correlations between serum reactivity and HLA determinants [5]. Positive correlations were considered statistically significant if the chi-square value was  $>6.8$  ( $p < 0.01$ ) and if there were no or few false-negative reactions. A statistically significant positive correlation could be interpreted as evidence that the serum contains a specific antibody against a given determinant. Many positive correlations, however, are secondary and they do not reflect the presence of a specific antibody. As an example, let us consider the a144tKr triplet that is expressed on HLA-A1, -A3, -A11, -A24, -A36, and -A80 molecules. Suppose a patient becomes allosensitized to the a144tKr triplet following exposure to HLA-A3. A monospecific anti-a144tKr antibody will exhibit a highly significant positive correlation (*i.e.*, no false-positive or false-negative reactions) with a144tKr. This is an example of a primary correlation. Such serum will often demonstrate significant correlations with other triplets found on HLA-A1, -A3, -A11, -A24, -A36, or -A80. Although an antibody against a HLA-A3 triplet (such as a163dT) is possible, this serum cannot contain antibodies against determinants absent from HLA-A3 mole-

HLA-	Position:	9	12	14	17	41	45	56	62	66	70	74	76	80	82	90	105	107	127	131	142	144	147	149	151	156	158	163	166	171	177	180	184	186	193	199	207	246	248	253	
A1	A*0101	F	sV	R	gR	A	kMe	G	Qe	rNm	aHs	D	An	gTL	IRg	D	P	G	N	R	I	tKr	W	aVh	vHa	R	V	R	Dg	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
A2	A*0201	F	sV	R	gR	A	Me	G	Ge	rKv	aHs	H	Vd	gTL	IRg	A	S	W	K	R	T	tKh	W	aAh	aHv	L	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	A	V	Qe	
A3	A*0301	F	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	IRg	A	S	G	N	R	I	tKr	W	aAh	aHe	L	A	dT	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
A11	A*1101	Y	sV	R	gR	A	Me	G	Qe	rNv	aQs	D	Vd	gTL	IRg	D	P	G	N	R	I	tKr	W	aAh	aHa	Q	A	R	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
A23	A*2301	S	sV	R	gR	A	Me	G	Ee	GKv	aHs	D	En	rla	aLr	A	S	G	K	R	I	tQr	W	aAr	aRv	L	A	T	Dg	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
A24	A*2402	S	sV	R	gR	A	Me	G	Ee	GKv	aHs	D	En	rla	aLr	A	S	G	K	R	I	tKr	W	aAh	aHv	Q	A	T	Dg	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
A25	A*2501	Y	sV	R	gR	A	Me	G	Rn	rNv	aHs	D	Es	rla	aLr	D	P	G	N	R	I	tQr	W	tAh	aHe	W	A	R	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Qe	
A26	A*2601	Y	sV	R	gR	A	Me	G	Rn	rNv	aHs	D	An	gTL	IRg	D	P	G	N	R	I	tQr	W	tAh	aHe	W	A	R	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Qe	
A29	A*2902	T	sV	R	gR	A	Me	G	Lq	rNv	aQs	D	An	gTL	IRg	A	S	G	N	R	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Qe	
A30	A*3001	S	sV	R	gS	A	Me	R	Qe	rNv	aQs	D	Vd	gTL	IRg	A	S	G	N	R	I	tQr	W	aAr	aRw	L	A	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ec	
A31	A*3101	T	sV	R	gR	A	Me	R	Qe	rNv	aHs	iD	Vd	gTL	IRg	A	S	G	N	R	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	dP	K	Av	A	S	S	V	Qe	
A32	A*3201	F	sV	R	gR	A	Me	G	Qe	rNv	aHs	D	Es	rla	aLr	A	P	G	R	N	R	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Qe
A33	A*3301	T	sV	R	gR	A	Me	G	Rn	rNv	aHs	iD	Vd	gTL	IRg	A	S	G	N	R	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	R	Av	A	S	S	V	Qe	
A34	A*3402	Y	sV	R	gR	A	Me	G	Rn	rKv	aQs	D	Vd	gTL	IRg	D	P	G	N	R	I	tQr	W	tAh	aHe	W	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Qe	
A36	A*3601	F	sV	R	gR	A	kMe	G	Qe	rNm	aHs	D	An	gTL	IRg	D	P	G	N	R	I	tKr	W	aVh	vHa	R	V	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ec	
A43	A*4301	Y	sV	R	gR	A	Me	G	Lq	rNv	aHs	D	An	gTL	IRg	D	P	G	N	R	I	tQr	W	tAh	aHe	W	A	R	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Qe	
A66	A*6601	Y	sV	R	gR	A	Me	G	Rn	rNv	aQs	D	Vd	gTL	IRg	D	P	G	N	R	I	tQr	W	tAh	aHe	W	A	R	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Qe	
A68	A*6801	Y	sV	R	gR	A	Me	G	Rn	rNv	aQs	D	Vd	gTL	IRg	A	S	G	K	R	T	tKh	W	aAh	aHv	W	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	vA	V	Qe	
A69	A*6901	Y	sV	R	gR	A	Me	G	Rn	rNv	aQs	D	Vd	gTL	IRg	A	S	W	K	R	T	tKh	W	aAh	aHv	L	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	A	V	Qe	
A74	A*7401	F	sV	R	gR	A	Me	G	Qe	rNv	aHs	D	Vd	gTL	IRg	A	P	G	N	R	I	tQr	W	aAr	aRv	L	A	T	Ew	Y	Et	Q	dA	K	Av	A	S	S	V	Qe	
A80	A*8001	F	sV	R	gR	A	Me	E	Ee	rNv	aHs	N	An	gTL	IRg	D	S	G	R	N	R	I	tKr	W	aAr	aRr	L	A	E	Dg	Y	Et	Q	dP	K	P	A	S	A	V	Ke
B7	B*0702	Y	sV	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNI	IRg	A	P	G	N	R	I	tQr	W	aAr	aRe	R	A	E	Ew	Y	Dk	E	dP	K	Pi	A	G	A	V	Ee	
B8	B*0801	D	aM	R	gR	A	Ee	G	Rn	qif	tNt	D	Es	rNI	IRg	A	P	G	N	R	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	dP	K	Pi	A	G	A	V	Ee	
B13	B*1302	Y	aM	R	gR	A	Te	G	Re	qls	tNt	Y	En	rTa	aLr	A	P	G	N	S	I	tQl	W	aAr	aRv	L	A	E	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
B18	B*1801	H	sV	R	gR	A	Te	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	T	Ew	H	Et	Q	dP	K	Pi	A	G	A	V	Ee	
B27	B*2705	H	sV	R	gR	A	Ee	G	Rn	qic	aKa	D	Ed	rTI	ILr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	E	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
B35	B*3501	Y	aM	R	gR	A	Te	G	Rn	qif	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	L	Ew	Y	Et	Q	dP	K	Pv	A	G	A	V	Ee	
B37	B*3701	H	sV	R	gR	A	Te	G	Re	qls	tNt	Y	Ed	rTI	ILr	A	P	G	N	S	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
B38	B*3801	Y	sV	R	gR	A	Ee	G	Rn	qic	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	T	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
B39	B*3901	Y	sV	R	gR	A	Ee	G	Rn	qic	tNt	D	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	L	T	T	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
B41	B*4101	H	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	R	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	dP	K	Pi	A	G	A	V	Ee	
B42	B*4201	Y	sV	R	gR	A	Ee	G	Rn	qly	aQa	D	Es	rNI	IRg	A	P	G	N	R	I	tQr	W	aAr	aRv	D	A	T	Ew	Y	Dt	E	dP	K	Pi	A	G	A	V	Ee	
B44	B*4402	Y	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	En	rTa	aLr	A	P	G	N	S	I	tQr	W	aAr	aRv	D	A	L	Es	Y	Et	Q	dP	K	Pi	V	G	A	V	Ee	
B45	B*4501	H	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRv	D	A	L	Es	Y	Et	Q	dP	K	Pi	A	G	A	V	Ec	
B46	B*4601	Y	aM	R	gR	A	Ma	G	Re	qKy	rQa	D	Vs	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	W	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ec	
B47	B*4701	Y	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	Ed	rTI	ILr	A	P	G	N	S	I	tQr	W	aAr	aRv	L	A	E	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ee	
B48	B*4801	Y	sV	R	gR	A	Ee	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	R	I	sQr	L	aAr	aRv	L	A	E	Ew	Y	Dk	E	dP	K	Pi	A	G	A	V	Ee	
B49	B*4901	H	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ec	
B50	B*5001	H	aM	R	gR	T	Ke	G	Re	qls	tNt	Y	Es	rNI	IRg	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	Y	Et	Q	dP	K	Pi	A	G	A	V	Ec	
B51	B*5101	Y	aM	R	gR	A	Te	G	Rn	qif	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	H	Et	Q	dP	K	Pv	A	G	A	V	Ec	
B52	B*5201	Y	aM	R	gR	A	Te	G	Re	qls	tNt	Y	En	rla	aLr	A	P	G	N	S	I	tQr	W	aAr	aRe	L	A	L	Ew	H	Et	Q	dP	K	Pv	A	G	A	V	Ec	
B53	B*5301	Y	aM	R	gR	A	Te	G	Rn	qif	tNt	Y	En	rl																											



**TABLE 2** Frequencies of positive and negative correlations for triplets that are polymorphic for one HLA locus but monomorphic for another HLA locus

Triplet	Monomorphic for locus	Number of cases	Number of positive correlations	% Positive correlations	Number of negative correlations	% Negative correlations
a56G	BC	1	0	0%	1	100%
a821Rg	C	1	0	0%	1	100%
a90A	B	7	0	0%	5	71%
107G	BC	3	0	0%	2	67%
a127N	BC	8	0	0%	4	50%
a142I	BC	6	0	0%	3	50%
a144tQr	C	29	2	7%	19	66%
a149aAr	B	38	3	8%	23	61%
a166Ew	C	2	0	0%	2	100%
a183P	B	14	1	7%	8	57%
a207G	BC	16	0	0%	7	44%
a246A	BC	4	0	0%	3	75%
a253E	B	16	0	0%	7	44%
b12sV	A	23	2	9%	20	87%
b41A	AC	7	0	0%	7	100%
b62Re	C	36	0	0%	25	70%
b821Rg	C	15	7	47%	8	53%
b131R	AC	35	9	26%	18	51%
b144tQr	C	2	0	0%	2	100%
b147W	A	2	0	0%	2	100%
b166Ew	C	1	0	0%	1	100%
b171Y	A	3	0	0%	3	100%
b177Et	A	11	1	9%	11	100%
b180Q	AC	11	1	9%	11	100%
b199A	AC	1	0	0%	1	100%
Total:		292	26	9%	194	66%

(Table 2). Twenty-three triplets exhibited no or few positive correlations and the frequencies of negative correlations were generally high. The overall incidence of positive correlations was 9% and the frequency of negative correlations was 66%.

Two triplets revealed rather high frequencies of positive correlations, but these appeared to be secondary correlations. The 821Rg triplet is polymorphic for HLA-B, but monomorphic for HLA-C. The leucine residue of b821Rg is shared with the neighboring b80rN1 triplet on Bw6-associated HLA-B antigens and all seven positive correlations of b821Rg appeared to be secondary to correlations with the polymorphic b80rN1.

The 131R triplet is polymorphic for HLA-B, but monomorphic for HLA-A and HLA-C. The b131R triplet is present on the Bw6-associated HLA-B7, -B8, -B41, -B42, -B48, -B60, -B61, -B73, and -B81 molecules. Nine cases (43%) characterized positive correlations of b131R, and six appeared to be secondary to positive correlations of b80rN1 found on all Bw6-associated HLA-B molecules. Eight positive correlations of b131R appeared to be secondary to positive correlations of the polymorphic b180E triplet present on HLA-B7, -B8, -B41, -B42, -B48, -B60, -B73, and -B81 molecules.

These findings support the concept that triplets that are polymorphic for one locus but monomorphic for another locus do not induce the production of specific antibodies in allosensitized patients.

The second group of interlocus matches dealt with 731 cases involving 15 triplets that are polymorphic at both HLA-A and HLA-B loci. Depending on the HLA type of the patient, a triplet on a mismatched HLA-A or HLA-B antigen could represent an interlocus match (428 cases) or a true mismatch (303 cases). Table 3 illustrates that a total of 163/303 (or 54%) of the interlocus triplet mismatches exhibited a positive correlation between serum reactivity and the particular triplet. In contrast, only 69/428 (or 16%) the interlocus triplet matches revealed a positive correlation ( $p < 0.0001$ ).

Most positive correlations for the interlocus triplet matches in Table 3 can be explained as secondary to correlations with other polymorphic triplets. As an example, HLA-A2 and HLA-B17 molecules have the same 62Ge triplet. In 24 cases, 62Ge was an interlocus match. Three patients typed as HLA-B17 but not HLA-A2 and, therefore, the 62Ge triplet on HLA-A2 (*i.e.*, a62Ge) was considered an interlocus match. For all three patients, the serum reactivity correlated strongly with the immuno-

**TABLE 3** Frequencies of positive and negative correlations for polymorphic triplets that are interlocus matches and interlocus mismatches

Triplet	HLA antigens that express triplet*	Interlocus matches			Interlocus mismatches		
		Total	Positive corr	% Positive corr	Total	Positive corr	% Positive corr
62Ge	A2,B17	24	6	25%	38	25	66%
62Rn	A10,A28,B7,B8,B14,B16,B22,B18,B35,B42,B51,B53,B59,B67,B71,B73, B75,B77,B81,B82	33	6	18%	11	4	36%
66rNm	A1,A36,B17,B63	19	7	37%	42	17	40%
74D	A1,A3,A11,A9,A10,A28,A29,A30,A32,A36,A43,A74,B7,B8,B14,B22,B39, B42,B46,B67,B73,B78,B81,B82	29	7	24%	2	1	50%
76En	A9,B5,B13,B17,B27,B38,B44,B49,B53,B59,B63,B77	28	9	32%	28	21	75%
76Es	A25,A32,B7,B8,B14,B18,B22,B35,B39,B40,B41,B42,B45,B48,B50,B62, B67,B71,B75,B76,B78,B81,B82	43	10	23%	13	7	54%
80rIa	A9,A25,A32,B5,B17,B38,B49,B53,B59,B63,B77	25	7	28%	33	23	70%
82aLr	A9,A25,A32,B5,B13,B17,B38,B44,B49,B53,B59,B63,B77	21	4	19%	26	20	77%
151aRv	A23,A29,A31,A32,A33,A74,B8,B12,B13,B16,B17,B18,B27,B35,B37,B41, B42,B47,B48,B53,B54,B56,B61,B67,B73,B81,B82	43	3	8%	6	3	50%
156L	A2,A3,A23,A29,A30,A31,A32,A33,A69,A74,A80,B5,B13,B14,B16,B17,B18, B21,B22,B27,B35,B47,B48,B53,B59,B60,B63,B67,B70,B73,B75,B77,B78,B81	21	0	0%	1	0	0%
156R	A1,A36,B7	24	6	25%	34	16	47%
156W	A10,A28,A34,A43,B46,B62,B76	24	1	4%	38	11	29%
163E	A80,B7,B13,B27,B47,B48,B60,B61,B73,B81	34	0	0%	28	17	61%
163T	A2,A9,A28,A29,A30,A31,A32,A33,A34,A36,A74,B8,B14,B16,B18,B37, B41,B42,B54,B55,B59,B67	42	2	5%	2	0	0%
193Pi	A1,A3,A9,A11,A30,A36,A80,B7,B8,B12,B13,B14,B15,B16,B18,B21,B22, B27,B37,B40,B41,B42,B46,B47,B48,B59,B67,B73,B81,B82	18	2	13%	1	0	0%
Total		428	69	16%	303	163	54%

\*The following broad specificities were used in this table: A9 = A23+A24; A10 = A25+A26+A34+A66; A28 = A68+A69; B = B51+B52; B12 = B44+B45; B14 = B64 + B65; B15 = B62+B63+B75+B76+B77; B16 = B38+B39; B17 = B57+B58; B21 = B49+B50; B22 = B54+B55+B56; B40 = B60+B61; B70 = B71+B72

genic a74H triplet that is unique for HLA-A2. The positive correlations with a62Ge appeared secondary to the correlations with a74H.

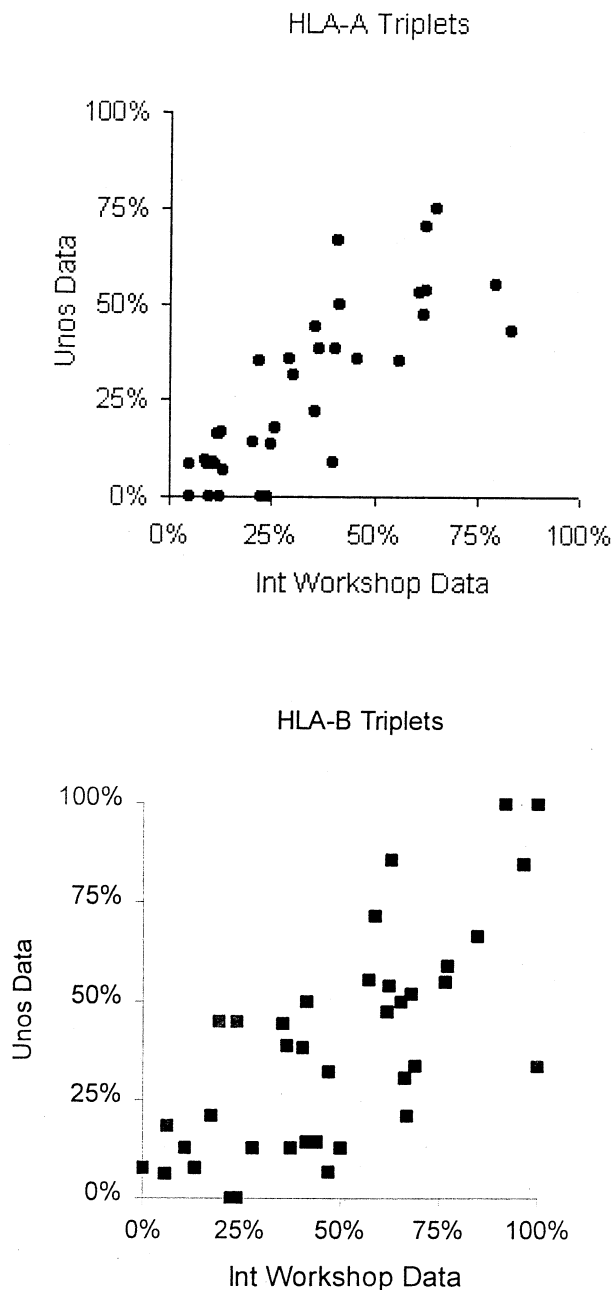
This type of analysis was done on all positive correlations between serum reactivity and interlocus triplet matches. Most of these correlations could be explained to be secondary correlations and they did not clearly demonstrate specific antibodies (data not shown). These findings are consistent with the concept that a polymorphic triplet on a mismatched HLA antigen cannot induce antibodies in patients whose HLA antigens have that triplet in the same sequence position.

#### Frequencies of Negative Correlations in Two Groups of High PRA Sera

Although most high PRA sera exhibit a fair number of positive correlations with triplet-associated epitopes, it remains uncertain how many are truly primary correlations due to the presence of specific antibodies. On the other hand, the finding of a significant negative correlation between serum reactivity and a cell panel marker provides more conclusive evidence albeit in relation to the absence of a specific antibody. Many negative corre-

lations found in this analysis pertain to mismatched HLA antigens. This means that none of the triplets on such antigens react with the patient's antibodies and, therefore, they are acceptable mismatches. This information has important implications because it may permit the identification of other mismatched HLA antigens with zero/acceptable triplet mismatches without further serum screening with additional panel cells [1,2].

The frequency of a negative correlation may provide information about the immunogenicity of a polymorphic triplet. A high frequency indicates that most patients do not produce specific antibodies to a given triplet and, therefore, such triplet cannot be very immunogenic. We have compared the frequencies of negative correlations in two groups of high PRA sera: (1) 62 sera had been screened by AHG methods during the 12th Workshop; and (2) 65 sera had been screened by CDC methods in UNOS Region 2 laboratories. Although these serum screens had been done under different test conditions, the results of this analysis demonstrated a rather high level of concordance between the frequencies of negative correlations for the polymorphic triplets on HLA-A and HLA-B antigens (Figure 2). These findings suggest that



**FIGURE 2** Frequencies of negative correlations for HLA-A and HLA-B triplets with PRA sera. Comparisons are between the 12th International Workshop ( $n = 62$ ) and UNOS Region 2 data ( $n = 65$ ).

these two separate studies yield reproducible data relevant to triplet immunogenicity.

#### Relative Immunogenicity of Mismatched Triplets

Table 4 illustrates the frequencies of negative and positive correlations for 79 polymorphic triplets following analysis of 12th Workshop sera screened by AHG and CDC. In order to estimate the relative immunogenicity

of a triplet we have calculated the ratio of the frequencies of positive and negative correlations. Triplets with high ratios seem to induce specific antibodies more frequently than triplets with low ratios. Typical examples of highly immunogenic triplets (ratio  $>2.0$ ) are a74H, a127K, a142T, ab62Ge (they are found on members of the A2 cross-reacting group [CREG] that includes HLA-A2, -A9, -A28, and -B17), and the Bw4-associated triplets ab82aLr and ab76En. Twenty-three triplets have ratios below 0.3 and they are listed at the bottom of Table 4. These triplets seem to have low immunogenicity and they might be viewed as permissible mismatches.

This analysis did not consider triplet polymorphisms at the HLA-C locus because no reliable HLA-C typing information was available for the patients and panel cells in the datasets. Several triplets such as 90D, 150aRe, and 156R are present on groups of HLA-C antigens and in such instances, a90D, b150aRe, and ab156R would be interlocus matches if the patient typed for one of these HLA-C antigens. Thus, we must expect for these triplets (they are marked with an asterisk) that the actual number of true mismatches would be smaller than the number of cases listed in Table 4, and that the relative immunogenicity ratios might be different from those described.

#### DISCUSSION

HLAMatchmaker considers the concept that each HLA antigen represents a string of multiple triplet-associated epitopes that can be recognized by specific alloantibodies. How do these antibodies interact with HLA antigens? Three-dimensional structures of different antigen-antibody complexes have revealed that up to six hypervariable loops (or complementarity determining regions [CDR]) of the antibody binding site make contact with a protein antigen [6, 7]. The contact area between antibody and antigen is about 700 to 800 square angstroms [8–10] and this is similar to the size of the HLA molecular surface seen from above the peptide-binding region and the alpha helices [11]. An epitope on any protein antigen contains a few critical residues that provide dominant contributions to the binding energy with one of the CDRs [12]. There are also many contact residues on the protein antigen 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, we believe 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

**TABLE 4** Immunogenicity estimations of polymorphic triplets

Triplet	Triplet-expressing HLA antigens	Number of patients	Percentage of negative correlations	Percentage of positive correlations	% Pos/ % Neg ratio
a66rKv	A2	85	4%	68%	19.3
a74H	A2	85	4%	68%	19.3
b80rTa	B13,B44	99	5%	71%	14.1
b199V	B44	108	5%	62%	13.3
a142T	A2,A28	70	6%	69%	12.2
a144tKh	A2,A28	70	6%	67%	11.7
a107W	A2,A69	83	6%	66%	10.9
ab62Ge	A2,B17	80	10%	66%	6.6
b166Es	B12,B82	103	11%	58%	5.4
a62Ee	A9,A80	97	9%	46%	5.0
a66gKv	A9	97	10%	44%	4.3
a151aHv	A2,A24,A28	61	15%	63%	4.2
a151aHa	A11	109	6%	19%	3.5
a127K	A2,A9,A28	51	18%	60%	3.4
a156Q	A11,A24	90	10%	30%	3.0
163dT	A3	98	13%	36%	2.7
b82aLr	A9,A25,A32,B5,B17,B38,B49,B53,B59,B63,B77	46	28%	77%	2.7
ab76En	A23,A24,B5,B13,B17,B38,B44,B49,B53,B59,B53,B77	50	28%	75%	2.7
b177Dk	B7,B48,B60,B81	88	22%	51%	2.4
a149aVh	A1,A36	106	15%	30%	2.0
a151vHa	A1,A36	106	15%	30%	2.0
a158V	A1,A36	106	15%	30%	2.0
a45kMe	A1,A36	106	15%	30%	2.0
b9D	B8	116	21%	39%	1.9
ab80rIa	A9,A25,A32,B5,B13,B17,B38,B44,B49,B53,B59,B63,B77	61	41%	70%	1.7
b163E	A80,B7,B13,B27,B40,B47,B48,B73,B81	68	38%	61%	1.6
a166Dg	A1,A9,A80	77	19%	30%	1.5
a144tKr	A1,A3,A11,A24,A36,A80	53	25%	35%	1.4
b41T	B12,B13,B21,B40,B41,B47	73	32%	43%	1.4
b80rTI	B27,B37,B47	116	17%	23%	1.3
b82ILr	B27,B37,B47	116	17%	23%	1.3
b76Ed	B27,B37,B47	116	17%	23%	1.3
b45Ke	B12,B21,B41,B40,B47	80	33%	42%	1.3
a163R	A1,A11,A25,A26,A43,A66	77	26%	33%	1.3
b70aQa	B7,B42,B22,B67,B76,B81,B82	86	33%	40%	1.2
ab66rNm	A1,A36,B17,B63	101	34%	40%	1.2
b66qIy	B7,B42,B22,B67,B76,B81,B82	86	34%	40%	1.2
ab76Es	A25,A32,B7,B8,B14,B18,B22,B35,B39,B40,B41,B42, B45,B48,B50,B62,B67,B70,B75,B78,B81,B82	24	50%	54%	1.1
a151aHe	A3,A10	76	32%	31%	1.0
b80rNL	B7,B8,B14,B18,B22,B39,B40,B41,B42,B45,B46,B48, B50,B62,B76,B70,B75,B76,B78,B81,B82	26	58%	53%	0.9
a9S	A9,A30	76	45%	40%	0.9
a149tAh	A10	106	19%	16%	0.9
a149aAh	A2,A3,A11,A24,A28	30	33%	29%	0.9
b180E	B7,B8,B41,B42,B48,B60,B81	75	49%	39%	0.8
b193Pv	B5,B35,B53,B58,B78	74	74%	50%	0.7
a76An	A1,A26,A29,A36,A43,A80	86	37%	25%	0.7
ab156R*	A1,A36,B7	77	70%	47%	0.7
ab156W*	A10,A43,A68,B46,B62,B76	83	46%	29%	0.6
a183A	A2,A10,A28,A29,A32,A43,A74	42	48%	29%	0.6
ab62Rn	A10,A28,B7,B8,B14,B16,B22,B18,B35,B42,B51,B53, B59,B67,B71,B73,B75,B77,B81,B82	66	72%	36%	0.5
a9T	A29,A21,A33	106	39%	18%	0.5
b66qIs	B12,B13,B18,B21,B37,B40,B41,B47,B48,B52,B62, B70,B72,B75,B77	57	70%	32%	0.5
a90D*	A1,A10,A11,A36,A43,A80	67	55%	24%	0.4

*(Continued)*

TABLE 4 Continued

Triplet	Triple-expressing HLA antigens	Number of patients	Percentage of negative correlations	Percentage of positive correlations	% Pos/ % Neg ratio
a207S	A2,A10,A28,A29,A31,A32,A33,A43,A74,A80	35	60%	24%	0.4
b45Ma	B13,B15,B46,B57	105	35%	13%	0.4
b177Dt	B8,B41,B42	108	44%	16%	0.4
a253Q	A2,A10,A28,A29,A31,A32,A33,A43,A74,A80	35	60%	18%	0.3
b156D	B8,B12,B37,B41,B42,B82	80	58%	16%	0.3
ab171H	A33,B5,B14,B18,B73,B78	90	56%	15%	0.3
a62Qe	A1,A3,A11,A30,A31,A32,A36,A74	45	51%	13%	0.2
a9F	A1,A2,A3,A32,A36,A74,A80	54	52%	13%	0.2
b66qIf	B8,B35,B51,B53,B59,B78	67	51%	12%	0.2
a70aQs	A3,11,A28,A29,A30,A34,A66	44	48%	11%	0.2
b74Y	B5,B12,B13,B15,B17,B18,B21,B35,B37,B38,B40,B41, B47,B48,B53,B59,B70	21	67%	15%	0.2
a246S	A10,A29,A31,A32,A33,A43,A74	77	58%	11%	0.2
b163L	B5,B12,B15,B17,B21,B35,B46,B53,B56,B70,B78	44	61%	12%	0.2
a56R	A30,A31	108	32%	6%	0.2
a66rNv	A3,A11,A10,A28,A29,A30,A31,A32,A33,A43,A74,A80	29	62%	11%	0.2
a76Vd	A2,A3,A11,A28,A30,A31,A33,A34,A66,A74	16	63%	11%	0.2
a70aHs	A1,A2,A9,A25,A26,A31,A32,A33,A36,A43,A74,A80	23	74%	9%	0.1
b45Ee	B7,B8,B14,B27,B38,B39,B42,B48,B55,B56,B59,B67, B70,B73,B81,B82	51	65%	8%	0.1
b150aRe*	B5,B7,B14,B15,B21,B46,B55,B70,B78	54	93%	11%	0.1
b45Te	B5,B18,B35,B37,B53,B58,B78	68	54%	6%	0.1
a193Av	A2,10,A28,A29,A31,A32,A33,A43,A74	35	60%	6%	0.1
b131S	B5,B13,B14,B15,B16,B17,B18,B21,B22,B27,B35,B37, B46,B47,B53,B59,B67,B70,B78,B82	20	90%	8%	0.1
b9H	B21,B27,B37,B40,B41,B45,B73,B18	84	69%	5%	0.1
b66qIc	B14,B16,B27,B71,B73	97	61%	4%	0.1
b12Am	B5,B8,B12,B13,B15,B17,B21,B22,B35,B41,B46,B47, B53,B59,B60,B70,B78,B82	13	69%	0%	0.0
b70tNt	B5,B8,B12,B13,B14,B16,B18,B21,B35,B37,B40,B41, B47,B48,B53,B59,B62,B70,B75,B76,B77,B78	13	100%	0%	0.0

\*Also expressed as HLA-C antigens.

alloantibodies against HLA and why their detection is so often technique-dependent. During the immunizing event, the patient develops an antibody to a given triplet present on the immunizing HLA antigen. This alloantibody may have one CDR (*e.g.*, CDR-H3 on the immunoglobulin heavy chain) that recognizes a polymorphic triplet, whereas the remaining CDRs interact with other parts of the immunizing HLA molecule to strengthen the binding between HLA and alloantibody. This interaction may lead to the release of sufficient energy to induce a conformational change in the Fc portion of the antibody molecule, thereby exposing the C1q receptor site and initiating the activation of the complement cascade needed for lymphocytotoxicity. If the triplet recognized by CDR-H3 of alloantibody is on another HLA molecule with a significantly different amino acid sequence than the immunizing HLA molecule, it seems possible that the binding of the remaining CDRs is not strong enough to expose the C1q receptor site needed for complement activation. In other words, the antibody will

bind to this HLA antigen on a lymphocyte surface, but no cytotoxicity is seen following addition of complement. This would be an example of the cytotoxicity-negative, adsorption-positive phenomenon (CYNAP) [13, 14]. In such an instance the addition of AHG might be necessary for the activation of the complement cascade leading to lymphocytotoxicity [15, 16]. Antibodies against immunoglobulin light chains are particularly effective in exposing C1q receptors on alloantibodies with CYNAP activity [16].

This report deals with an analysis of complement-dependent, lymphocytotoxicity-based serum screening data to determine antibody reactivity against polymorphic triplets. There were two goals. The first goal was to verify the concept that sensitized patients cannot produce antibodies to triplets are present in the same sequence location of any of the patient's own HLA molecules. This was done with standard chi-square statistical methods to determine significant correlations between serum reactivity and the presence of specific triplets assigned to the

HLA-typed cell panels. Although positive correlations are generally interpreted as evidence for specific antibodies, it became apparent that with high PRA sera, this statistical approach has its limitations because many positive correlations seemed secondary and they did not reflect antibody specificity to a given triplet. In the group of 62 12th Workshop sera screened by both CDC and the more sensitive AHG methods, the interlocus triplet matches exhibited low frequencies of positive correlations and most of them seemed to be secondary.

The assignment of triplets to the HLA antigens of patients and panel cells was based on amino acid sequence information of what was estimated to be the most common molecularly defined allele of each serologically defined HLA antigen. It is possible that many of these HLA antigens represented different molecularly defined alleles. Although the triplet variability is generally low among molecular HLA antigen subtypes (unpublished observations), we must conclude that in this study, the triplet assignments were not always accurate.

In spite of uncertainties about triplet assignments and limitations of the chi-square statistics for high PRA sera, such as interpretations of positive correlations, these findings are consistent with the concept that interlocus triplet matches do not produce antibodies in allosensitized patients. Moreover, recent studies by other investigators have reported that HLAMatchmaker-predicted compatibility at the triplet level corresponds with serum screening and crossmatching results obtained by flow cytometric methods [17, 18].

Class I HLA matching at the triplet level benefits kidney transplant outcome [19]. In two large databases of zero HLA-DR mismatched kidney transplants, we have found that HLA-A,B mismatches that are matched at the triplet level have very similar graft survival rates as the conventional zero HLA-A,B antigen mismatches. The findings support the clinical validity of the HLA-Matchmaker algorithm.

The second goal of this study addressed the relative immunogenicity of mismatched triplets. By determining the ratios of frequencies of positive and negative correlations between serum reactivity and the presence of unshared triplets on panel cells, we have identified triplets with high or low immunogenicity. This type of information could be applied to new molecularly-based strategies to determine donor-recipient compatibility in transplantation and matched platelet transfusions of refractory thrombocytopenic patients. Claas and coworkers [20, 21] have reported that the serum screening with panel cells selectively mismatched for one HLA-A or B antigen permits the identification of acceptable HLA mismatches for highly sensitized patients. Mismatching for triplets with low immunogenicity provides further

opportunities for identifying donors with acceptable HLA mismatches for highly sensitized patients.

The immunogenicity data in this article must be considered preliminary because the triplet assignments were based on serologically defined HLA antigens, rather than the more accurate DNA-typed alleles, and because this analysis did not consider triplet polymorphisms of HLA-C alleles, which would have permitted a better assessment that a triplet was a match or mismatch. Nevertheless, two recent studies in other laboratories have incorporated our data on relative immunogenicity of triplets and both have demonstrated that the number of highly immunogenic triplets can serve as a predictor of the final crossmatch outcome [17, 18].

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