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Identification of epitopes on HLA-DRB alleles reacting with antibodies in sera from women sensitized during pregnancy

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ABSTRACT

This report describes a HLAMatchmaker-based antibody analysis of post-pregnancy sera with antibodies against child-specific HLA-DR epitopes. These sera were reactive in IgG-binding assays with single allele bead (SAB) panels on a Luminex platform. The antibody specificity analysis focused on DRB epitopes that have been recorded in the International HLA Epitope Registry (http://www.epregistry.com.br) as experimentally verified with informative antibodies but we also considered other eplets that predict potential epitopes.

The SAB panel has in several instances two or more alleles corresponding the same serologically defined DR antigen and we selected six sera were with different reactivity patterns with DR1, DR4, DR13 and/or DR52 alleles. We demonstrate here how amino acid differences between these alleles can provide useful information in the determination of new epitope specificities of antibodies in these sera. Eight newly antibody-verified epitopes were identified including three that correspond to eplets paired with self-residue configurations. Epitope specificity information appears to be useful in the prediction of mismatch acceptability of non-SAB alleles within serological DR antigen groups.

1. Introduction

There is now increasing acceptance of the concept that mismatch acceptability for sensitized transplant patients should be based on HLA epitopes recognized by antibodies and involve HLA typing at high-resolution rather than antigen levels [1]. HLAMatchmaker is an algorithm designed to predict HLA epitopes by molecular structural modeling and amino acid sequence comparisons between HLA alleles. It considers each allele as a series of small configurations of polymorphic residues referred to as eplets as essential components of HLA epitopes. The website-based International Registry of HLA epitopes http://www.epregistry.com.br describes the repertoires of HLA-ABC, -DRDQDP and -MICA eplets [2]. An important question is which eplets correspond to actual epitopes specifically recognized by HLA antibodies. Three publications describe antibody-verified epitopes recorded so far in the HLA Epitope Registry [3–5].

Pregnancy offers an attractive model to study the antibody responses to mismatched HLA epitopes which can be readily determined from the HLA types of mother and child. The goal of such studies is to determine how antibody reactivity with alleles in a HLA panel correlates with the presence of child-specific epitopes which have already been recorded as antibody-verified in the HLA Epitope Registry or other epitopes with distinct amino acid residue configurations. Such epitopes might be considered candidates to be added to the repertoire of antibody-verified epitopes.

This report describes a HLAMatchmaker analysis of pregnancy sera with DRB antibodies and tested in IgG-binding assays with single allele bead (SAB) panels on a Luminex platform. The SAB panel has in several instances two or more alleles corresponding the same serologically defined DR antigen and certain sera exhibit different reactivity patterns with such alleles. We postulate that amino acid differences between these alleles provide useful information in the determination of epitope specificities of antibodies. We describe here an epitope specificity analysis of antibodies of six sera with different reactivity patterns with alleles corresponding to DR1, DR4, DR13 and/or DR52 antigens. This epitope

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specificity information can be used in the prediction of mismatch acceptability of non-SAB alleles.

2. Methods

2.1. Populations and HLA typing and antibody testing methods

This study was approved by the local ethics committee and written informed consent was obtained from women enrolled in the study and giving full-term live birth at the University Hospital Basel between September 2009 and April 2011 [6]. A blood sample was drawn from the mother between day 1 and 4 after delivery for high-resolution HLA A/B/C/DRB1 typing and antibody testing. Cord blood of the child was obtained immediately after delivery for high-resolution HLA A/B/C/DRB1 typing. This report addresses antibody specificities against not only DRB1 epitopes but also DRB3, DRB4 and DRB5 epitopes. Although mother and child had been typed for only 4-digit DRB1 we assigned predicted DRB3/4/5 types from well-known strong associations within DRB haplotypes as reported elsewhere [7,8].

HLA antibody testing was done with single HLA antigen beads (SAB) for class I (iBeads, lot 8; One Lambda, ThermoFischer) and normalized mean fluorescence intensity (MFI) values were determined for each bead as previously described [6]. Determinations of cut-off MFI values between negative and positive reactions of alleles in the SAB panel were based on mean MFI values for the self-alleles of the mother; such values reflect true non-reactivity. Any other allele in the SAB panel with a MFI more than three standard deviations above the mean value with self can be statistically considered as being significantly higher and this criterion was applied to establish cut-off MFI values. Sera were considered to have child-reactive DRB-antibodies if any of the reactive alleles in the SAB panel corresponded with a paternal allele of the child.

2.2. HLAMatchmaker analysis

The newly designed www.HLAMatchmaker.net website has several updated epitope-based antibody analysis programs. All are in Microsoft Excel format and the following information can be readily entered: (1) the composition of the HLA-typed SAB panel, (2) 4-digit HLA types of the antibody producer and the immunizer if possible, and (3) the MFI values of the SAB panel can be copied from the CSV files in the SAB manufacturer's software stored in the Luminex equipment. The program automatically calculates the mean MFI value plus three standard deviations for the self-alleles of the antibody producer and this information can be used to determine the cut-off MFI value between negative and positive reactivity. Upon entering the cut-off MFI value, the program automatically identifies the reactive SAB alleles and their mismatched epitopes.

The antibody analysis programs consider two groups of epitopes: (1) epitopes that have been experimentally verified with informative antibodies, they are defined by eplets or eplet pairs and (2) other eplets in the SAB panel which should be considered as theoretical predictions of epitopes but might become antibody-verified if informative data with reactive alleles have been generated. Such newly identified epitopes will be recorded in the HLA Epitope Registry with the goal of establishing complete repertoires of epitopes recognized by antibodies.

The class II epitope analysis program addresses the reactivity the entire SAB panel. There are separate sheets for DRB1/3/4/5, DQA/DQB and DPA/DPB on which the SAB panel reactivity can be sorted according to immunizer-specific versus third-party-specific and antibody-verified epitopes versus other eplets. Moreover, the program has a list of non-SAB alleles that have or do not have epitopes shared with the reactive SAB alleles; this information is useful to determine mismatch acceptability of such alleles.

The SAB panel has in several instances two or more alleles corresponding the same serologically defined DR antigen and we noted that certain sera reacted differently with such alleles. We postulat that these differences can be explained with distinct amino acid differences between alleles; Table 1 shows which residues must be considered for the alleles in the current SAB panel. Moreover, reactive alleles corresponding to a given DRB antigen must share distinct residue configurations with the immunizing DRB allele whereas non-reactive alleles have different configurations. This report describes the reactivity differences of six sera with alleles corresponding to DR1, DR4, DR13 and/or DR52.

3. Results

3.1. Serum #22

This serum came from a woman who typed as DRB1*08:01, 15:01; DRB5*01:01 who after a first pregnancy had antibodies induced by the child's DRB1*01:01 (Table 2). The allele reactivity pattern did not reveal any antibody-verified epitope specificity. In the SAB panel, alleles in the DR1 and DR4 groups showed different reactivity patterns that were associated with amino acid differences shown in Table 1. The reactive DRB1*01:01, DRB1*01:02, DRB1*04:01, DRB1*04:03, DRB1*04:04 and DRB1*04:05 share the 67L and 70Q residues (or the 67LQ eplet) whereas the non-reactive DRB1*01:03 and DRB1*04:02 have 67ID. These findings suggest that 67LQ contributes to the epitope recognized by antibodies in #22. This is non-self configuration because the DRB alleles of the antibody producer have different configurations in these sequence positions: DRB1*08:01 (67FD), DRB1*15:01 (67IQ) and DRB5*01:01 (67FD).

All three DR52 alleles have also 67LQ but only DRB*02:02 was reactive. This means that the epitope corresponding to 67LQ must have an additional residue configuration present on DRB3*02:02 but not on the other two DR52 alleles. Such residue configuration must be shared with the immunizing DRB1*01:01 and the other reactive 67LQ-carrying alleles. Only residue 60Y meets this requirement and it should be noted that this is a self-residue present on all alleles of the antibody producer. Accordingly, antibodies in #22 are specific for an epitope defined by 67LQ paired with self-60Y; this epitope has been annotated as 67LQ+s60Y. In contrast, the non-reactive DRB3*01:01 and DRB3*03:01 have 67LQ paired with 60S.

Table 2 shows also that DRB1*10:01, DRB4*01:01, and DRB4*01:02 have low but still significantly positive MFI values (mean: 560 ± 223 versus 11 ± 16 for 21 negative alleles). They have the 67LR+s60Y configuration which apparently has some weak cross-reactivity with the 67LQ+s60Y epitope. The 67LR-carrying allele DRB1*14:01 was non-reactive and has 60H rather than 60Y. Similarly, the weakly reactive DRB1*09:02 (MFI = 433) has the weakly cross-reactive 67FR+s60Y but the 67FR+60H-carrying DRB1*09:01 (MFI = 48) was non-reactive.

Altogether, serum #22 has antibodies specific for an epitope defined by the 67LQ eplet paired with a self-residue 60Y expressed on the molecular surface about 6 Å away, a sufficient distance for contact by two separate CDRs of antibody. Table 2 shows the molecular model. This epitope has been annotated as 67LQ+s60Y. A substitution of 70Q by 70R within the eplet permitted a very low level of cross-reactivity with antibody.

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Table 1
Amino acid residue differences between alleles corresponding to serological defined antigens in the SAB panel used for serum testing.

Allele	Residue differences						Allele	Residue differences															
DR1							DR13																
DRB1*01:01	67L	70Q	71R	85V	86G		DRB1*13:01	32H	37N	47F	57D	71E	86V										
DRB1*01:02	67L	70Q	71R	85A	86V		DRB1*13:03	32Y	37Y	47Y	57S	71K	86G										
DRB1*01:03	67I	70D	71E	85V	86G																		
							DR15																
DR3							DRB1*15:01	30Y	86V														
DRB1*03:01	26Y	28D	47F	86V			DRB1*15:02	30Y	86G														
DRB1*03:02	26F	28E	47Y	86G			DRB1*15:03	30H	86V														
DR4							DR16																
DRB1*04:01	57D	67L	70Q	71K	74A	86G	DRB1*16:01	67F															
DRB1*04:02	57D	67I	70D	71E	74A	86V	DRB1*16:02	67L															
DRB1*04:03	57D	67L	70Q	71R	74E	86V																	
DRB1*04:04	57D	67L	70Q	71R	74A	86V	DR51																
DRB1*04:05	57S	67L	70Q	71R	74A	86G	DRB5*01:01	6R	30D	37D	38L	67F	70D	71R	85V	86G	135S						
							DRB5*02:02	6C	30G	37N	38V	67I	70Q	71A	85A	86V	135G						
DR9																							
DRB1*09:01	57V	60S					DR52																
DRB1*09:02	57D	60Y					DRB3*01:01	11R	26Y	28D	30Y	37F	38L	40F	51T	57V	60S	74R	86G	140A	149Q	183A	189R
							DRB3*02:02	11L	26F	28E	30H	37Y	38A	40Y	51R	57D	60Y	74Q	86G	140A	149Q	183P	189S
DR11							DRB3*03:01	11L	26F	28E	30Y	37F	38V	40F	51T	57V	60S	74Q	86V	140T	149H	183P	189R
DRB1*11:01	86G																						
DRB1*11:04	86V						DR53																
							DRB4*01:01	135S															
DR12							DRB4*01:03	135G															
DRB1*12:01	67I																						
DRB1*12:02	67F																						

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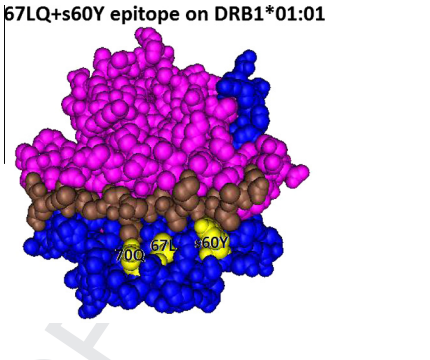
Table 2
Serum #22 has antibodies specific for an epitope defined by 67LQ paired with self 60Y.

Serum #22 Antibody producer: DRB1*08:01,*15:01; DRB5*01:01,-			
DRB allele		MFI	Ep1 Eplet pair*
DRB1*01:01	Immunizer	10,185	67LQ+s60Y
DRB1*01:02		13,466	67LQ+s60Y
DRB1*01:03		1	(67ID+s60Y)
DRB1*04:01		11,168	67LQ+s60Y
DRB1*04:02		1	(67ID+s60Y)
DRB1*04:03		5050	67LQ+s60Y
DRB1*04:04		13,585	67LQ+s60Y
DRB1*04:05		9535	67LQ+s60Y
DRB1*03:01		6523	67LQ+s60Y
DRB1*03:02		5337	67LQ+s60Y
DRB3*02:02		10,496	67LQ+s60Y
DRB3*01:01		1	(67LQ+60S)
DRB3*03:01		1	(67LQ+60S)
DRB1*10:01		309	(67LR+s60Y)
DRB4*01:01		735	(67LR+s60Y)
DRB4*01:03		637	(67LR+s60Y)
DRB1*14:01		26	(67LR+60H)
DRB1*09:02		433	(67FR+s60Y)
DRB1*09:01		48	(67FR+60H)
Negative alleles ($N = 21$)		11 ± 16	



The 67LQ eplet is paired with a self-residue 60Y expressed on the molecular surface about 6 Å away

* Non-reactive pairs are between parentheses.

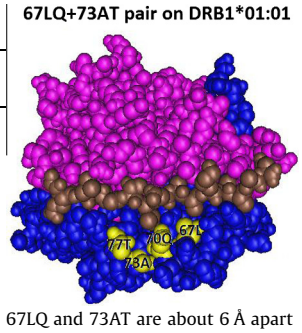
3.2. Serum #93

This serum came from a woman after a second pregnancy. She typed as DRB1*13:01, 15:01; DRB3*01:01, DRB5*01:01 and had antibodies induced by the child's DRB1*01:01 (Table 3). Reactive alleles share with DRB1*01:01 the antibody-verified 96ES$_2$ and an epitope defined by 13FE with DRB1*01:01; the 13FE-carrying DR9 alleles are informative. In contrast, the remaining reactive alleles did not share any mismatched antibody-verified epitope with the immunizing allele.

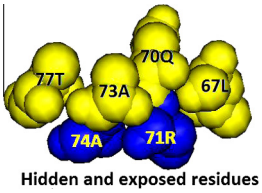
Four 67LQ-carrying DR4 alleles were reactive but the 67ID-carrying DRB1*04:02 was non-reactive. The immunizing DRB1*01:01 has also 67LQ which suggests that these residues contribute to the epitope recognized by antibodies in #93. However, both DRB1*03 and all three DRB3 alleles have also 67LQ but they were non-reactive. This suggests that the epitope requires another amino residue configuration that must be shared with the immunizing DRB1*01:01 and the reactive 67LQ-carrying alleles. The configuration of 73A and 77T meets this requirement because the non-reactive 67LQ-carrying DRB1*03 and DRB3 alleles have 73G and

Table 3
Serum #93 has antibodies specific for the antibody-verified 96ES$_2$ and two epitopes defined by 13FE and 67LQ or 67LR paired with self-73AT, respectively.

Serum #93 Antibody producer: DRB1*13:01, *15:01; DRB3*01:01, DRB5*01:01						
DRB allele		MFI	Ep1 AbVer	Ep2 Eplet	Ep3 Eplet pair*	Other residues in Ep3
DRB1*01:01	Immunizer	10,020	96ES$_2$	13FE	s67LQ+s73AT	71R 74A
DRB1*01:03		16,163	96ES$_2$	13FE		
DRB1*01:02		12,638	96ES$_2$	13FE	s67LQ+s73AT	71R 74A
DRB1*10:01		10,967		13FE	67LR+s73AT	71R 74A
DRB1*09:02		4164		13FE		
DRB1*09:01		2834		13FE		
DRB1*04:04		15,120			s67LQ+s73AT	71R 74A
DRB1*04:05		12,569			s67LQ+s73AT	71R 74A
DRB1*04:01		5152			s67LQ+s73AT	71K 74A
DRB1*04:03		4223			s67LQ+s73AT	71R 74E
DRB1*04:02		1			(67ID+s73AT)	
DRB1*03:01		65			(s67LQ+73GN)	
DRB1*03:02		93			(s67LQ+73GN)	
DRB3*02:02		1			(s67LQ+73GN)	
DRB3*03:01		1			(s67LQ+73GN)	
DRB3*01:01	Self	1			(s67LQ+73GN)	
DRB1*14:01		7776			67LR+s73AT	71R 74E
DRB4*01:01		7412			67LR+s73AT	71R 74E
DRB4*01:03		8625			67LR+s73AT	71R 74E
DRB5*02:02		702				
Negative alleles ($N = 20$)		33 ± 51				
Positive control		8453				



67LQ and 73AT are about 6 Å apart



* Non-reactive pairs are between parentheses.

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77N. Accordingly, #93 has antibodies reacting with a third epitope defined by 67LQ paired with 73AT.

Serum #93 reacted also with DRB1*14:01, DRB4*01:01 and DRB4*01:03 alleles which have 67LR paired with 73AT; There are two possible explanations for this reactivity. Since this serum was collected after a second pregnancy, it is possible that the epitope shared by these reactive alleles is recognized by antibodies induced by a different HLA mismatch during the first pregnancy. Another and perhaps more likely explanation is that the 67LQ +73AT epitope allows a substitution of glutamic acid by arginine in sequence position 70 but it leads to slightly lower MFI values. This would mean a serological cross-reactivity between 67LQ +73AT and 67LR+73AT.

DRB1*04:04 (MFI = 15,210) and DRB1*04:05 (MFI = 12,569) were more reactive than DRB1*04:01 (MFI = 5152) and DRB1*04:03 (MFI = 4223). DRB1*04:04 and DRB1*04:05 share nearby residues 71R and 74A with the immunizing DRB1*01:01 whereas DRB1*04:01 has 71K and 74A and DRB1*04:03, DRB1*14:01, DRB4*01:01 and DRB4*01:03 has 71R and 74E. Sequence positions 71 and 74 are not well exposed on the molecular surface and K/R and E/A substitutions might explain the lower MFI values.

For the epitope defined by the 67LQ+73AT pair it should be noted 67LQ is a self-eplet on the antibody producer's DRB3*01:01 and that 73AT is a self-eplet on the antibody producer's DRB1*13:01, DRB1*15:01 and DRB5*01:01. Accordingly, this epitope is annotated as s67LQ+s73AT. 67LQ and 73AT are about 6 Å apart, a sufficient distance for contact by two separate CDRs of antibody. The molecular model in Table 3 shows also the nearby locations of residue positions 71 and 74 below.

Serum #93 reacted weakly with DRB5*02:02 (MFI = 702) but not with the self-DRB5*01:01 of the antibody producer (MFI = 1). Three residue differences for DRB5*02:02 (38V, 70Q and 135D) are shared with the immunizing DRB1*01:01 but while it is possible that the DRB5*02:02 reactivity reflects antibodies induced by a mismatch during a previous pregnancy we could not identify a distinct epitope.

3.3. Serum #223

This serum was collected after a third pregnancy and had antibodies reacting with the DRB1*15:01/DRB5*01:01 haplotype of the child (Table 4). Its specificity corresponded to the antibody-verified $142M_3$ presented by DRB1*15:01 and shared with the other DRB1*15 and DRB1*16 alleles and the antibody-verified 108T on DRB5*01:01 and DRB5*02:02. The two DRB1*09 alleles had very low MFI values; they share 28H with the immunizing DRB5*01:01.

The reactivity patterns with DRB1*01 and DRB1*04 alleles were opposite than those with serum #22. It reacted with DRB1*01:03 and DRB1*04:02 but not with the other DRB1*01 and DRB1*04 alleles in the SAB panel. These reactive alleles share 70D with the immunizing DRB5*01:01 and all other 70D-carrying alleles such as DRB1*07:01, DRB1*11:01, DRB1*12:01 and DRB1*13:01 showed high MFI values. The non-reactive DR1 and DR4 alleles have 70Q and this suggested that #223 had antibodies specific for a fourth epitope defined by the 70D eplet.

3.4. Serum #384

This first pregnancy serum had antibodies induced by the DRB1*11:04/DRB3*02:02 haplotype and showed high MFI values with the antibody-verified $51R_2$ of the child's DRB3*02:02 and the antibody-verified 57DE epitope of the child's DRB1*11:04 (Table 5). Although the antibody producer typed as DRB1*13:01, her serum reacted with DRB1*13:03 which has six residue differences with DRB1*13:01 (Table 1) but only 32Y and 37Y are shared with the immunizing DRB1*11:04. This corresponds to two eplets in the epitope registry: 31FY and 37Y whereas the antibody producer's DRB1*13:01 and DRB3*01:01 have 31FH and 37F. All five DR4 alleles and DRB1*08:01 were reactive (MFI = 4383 ± 767) and they share 31FY+37Y. However, the non-reactive DR16 alleles carry 31IY+37Y and the non-reactive DRB4 and DRB1*15 alleles have 31FY+37S. This suggests that the epitope recognized by #384 is defined by 31FY+37Y. The 31FY+37F-carrying DRB1*07:01 was less reactive (MFI = 1504) but it is possible that the 31FY+37Y-specific antibodies in #384 cross-reacted with 31FY+37F (Table 5).

Based on the DRB eplet annotations in the International HLA Epitope Registry, this epitope is epitope determined by the combination of 31FY and 37Y. Both eplets are nonself and since they are less than 3.5 Å apart it seems likely that these residues comprise a new eplet annotated as 31FYY rather than the 31FY+37Y pair.

Table 4
Serum #223 has antibodies specific for four epitopes: the antibody-verified $142M_3$, and 108T and two epitopes defined by 28H and 70D, respectively.

Serum #223 Antibody producer: DRB1*01:01,*03:01; DRB3*01:01,-						
DRB allele	ImDRB	MFI	Ep1 VerEp	Ep2 VerEp	Ep3 Eplet	Ep4 Eplet*
DRB1*15:01	Immunizer	8962	$142M_3$			
DRB1*15:02		9726	$142M_3$			
DRB1*15:03		10,559	$142M_3$			
DRB1*16:02		13,492	$142M_3$			70D
DRB1*16:01		12,963	$142M_3$			70D
DRB5*01:01	Immunizer	13,021		108T	28H	70D
DRB5*02:02		12,887		108T	28H	
DRB1*09:01		294			28H	
DRB1*09:02		455			28H	
DRB1*01:03		7608				70D
DRB1*01:01		1				(70Q)
DRB1*01:02		1				(70Q)
DRB1*04:02		8257				70D
DRB1*04:01		16				(70Q)
DRB1*04:03		1				(70Q)
DRB1*04:04		1				(70Q)
DRB1*04:05		43				(70Q)
DRB1*07:01		6110				70D
DRB1*08:01		9254				70D
DRB1*11:01		9710				70D
DRB1*11:04		9923				70D
DRB1*12:01		12,545				70D
DRB1*12:02		10,308				70D
DRB1*13:01		11,480				70D
DRB1*13:03		10,080				70D
Negative alleles ($N = 14$)		36 ± 47				
Positive control		6390				

* Non-involved residues are between parentheses.

3.5. Serum #178

This serum was collected after a third pregnancy and had antibodies induced by DRB1*11:04/DRB3*02:02 haplotype and its reactivity pattern included specific recognition of the antibody-verified $51R_2$ and 57DE epitopes (Table 6). The immunizing DRB1*11:04 presented an epitope that was similar to 31FYY (or 31FY+37Y) recognized by #384 with two exceptions. First, the 31FY+37F-carrying DRB1*07:01 gave a negative reaction. Second, serum #178 reacted strongly with DRB1*13:03 (MFI = 12,264) and DRB1*08:01 (MFI = 11,389) but less well with the DRB1*04 alleles (MFI = 2601 ± 670). The strongly reactive alleles share nearby 10Y, 11S, 12T (all are hidden) and 33N with the immunizing DRB1*11:04 whereas the less reactive DR4 alleles have 10Q, 11V, 12K and 33H which appear to have a slightly negative effect

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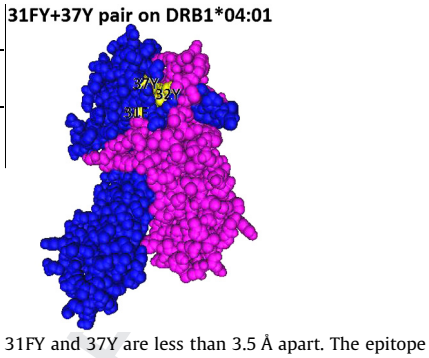
Table 5
Serum #384 has antibodies specific for the antibody-verified 51R2 and 57DE epitopes and an epitope defined by 31FY+37Y.

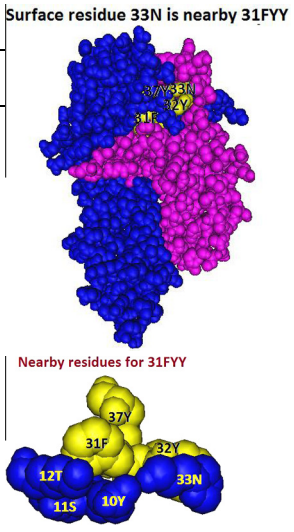
Serum #384 Antibody producer: DRB1*13:01,-; DRB3*01:01,-					
DRB allele		MFI	Ep1 AbVer	Ep2 AbVer	Ep3 Eplet pair*
DRB3*02:02	Immunizer	11,468	$51R_2$		(31FH+37Y)
DRB1*11:04	Immunizer	11,215		57DE	31FY+37Y
DRB1*11:01		10,331		57DE	31FY+37Y
DRB1*13:03		4704			31FY+37Y
DRB1*13:01	Self	1			(31FH+37N)
DRB3*01:01	Self	1			(31FH+37F)
DRB3*03:01		1			(31FH+37F)
DRB1*08:01		3140			31FY+37Y
DRB1*04:01		4806			31FY+37Y
DRB1*04:02		4308			31FY+37Y
DRB1*04:03		4551			31FY+37Y
DRB1*04:04		5425			31FY+37Y
DRB1*04:05		4066			31FY+37Y
DRB1*16:01		85			(31IY+37Y)
DRB1*16:02		1			(31IY+37Y)
DRB1*07:01		1504			31FY+37F
DRB4*01:01		1			(31FY+37S)
DRB4*01:03		23			(31FY+37S)
DRB1*15:01		28			(31FY+37S)
DRB1*15:02		21			(31FY+37S)
DRB1*15:03		16			(31FY+37S)
Negative alleles ($N = 23$)		13 ± 28			
Positive control		6808			



Non-involved pairs are between parentheses.
* The 31FY+37Y pair will be annotated as 31FYY.

Table 6
Serum #178 has antibodies specific for the antibody-verified 51R2 and 57DE epitopes and a third epitope annotated as 31FYY.

Serum #178 Antibody producer: DRB1*13:01,15:01; DRB3*01:01,DRB5*01:01						
DRB allele		MFI	Ep1 AbVer	Ep2 AbVer	Ep3 Eplet pair*	Other residues in Ep3
DRB3*02:02	Immunizer	13,641	$51R_2$		(31FH+37Y)	
DRB1*11:04	Immunizer	15,161		57DE	31FYY	10Y 11S 12T 33N
DRB1*11:01		14,486		57DE	31FYY	10Y 11S 12T 33N
DRB1*13:03		12,264			31FYY	10Y 11S 12T 33N
DRB1*13:01	Self	25			(31FH+37N)	
DRB1*08:01		11,339			31FYY	10Y 11S 12T 33N
DRB1*04:01		2383			31FYY	10Q 11V 12K 33H
DRB1*04:02		2940			31FYY	10Q 11V 12K 33H
DRB1*04:03		2927			31FYY	10Q 11V 12K 33H
DRB1*04:04		3223			31FYY	10Q 11V 12K 33H
DRB1*04:05		1533			31FYY	10Q 11V 12K 33H
DRB1*07:01		120			(31FY+37F)	
DRB1*15:01	Self	39			(31FY+37S)	
DRB1*15:02		29			(31FY+37S)	
DRB1*15:03		41			(31FY+37S)	
DRB1*16:01		13			(31FY+37S)	
DRB1*16:02		1			(31FY+37S)	
DRB3*01:01	Self	1			(31FH+37F)	
DRB3*03:01		1			(31FH+37F)	
DRB4*01:01		127			(31IY+37Y)	
DRB4*01:03		67			(31IY+37Y)	
Negative alleles ($N = 24$)		82 ± 71				
Positive control		6943				



* 31FYY corresponds to 31FY+37Y non-reactive pairs are between parentheses.

on the expression of the 31FYY epitope. The molecular model in Table 6 shows the location of the 31FYY epitope and the residues in nearby positions 10, 11, 12 and 33.

3.6. Serum #46

This serum was collected after a second pregnancy and had antibodies that reacted with the DRB1*13:01 of the child; the highly reactive alleles shared the antibody-verified 96HK epitope (Table 7). There were also antibodies to an epitope defined by the 47F eplet presented by the immunizing DRB1*13:01 and the reactivity of DRB1*15 alleles was informative for 47F specificity determination.

There was a similar reactivity with DRB1*01 and DRB1*04 alleles as #223 namely that that only DRB1*01:03 and DRB1*04:02 were reactive. It should be noted that the antibody producer typed

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as DRB1*04:05 which as expected, gave a negative MFI value. The reactive DRB1*01:03 and DRB1*04:02 share 70DA with the immunizing DRB1*13:01 of the child; the non-reactive DR4 have 70QA. High MFI values were seen for all remaining 70DA-carrying alleles DRB1*16 and DRB5*01:01. These findings suggest that #46 recognizes a third epitope defined by 70DA and presented by DRB1*11:04.

Serum #46 reacted strongly (MFI = 10,164) with the child's DRB3*02:02 which has a unique antibody-verified $51R_2$ epitope and reacted also with DRB3*01:01 (MFI = 5133) which shares an epitope defined by 77N+85VG whereby 77N is nonself and 85VG is self. In contrast, the 77N+85VV-carrying DRB3*03:01 was nonreactive (MFI = 1). This reactivity pattern suggests the presence of antibodies against an epitope defined by the 77N+s85VG pair. As illustrated in the molecular model in Table 7, these residues are 9 Å apart, a sufficient distance for contact by two separate CDRs of antibody.

3.7. Acceptable mismatches

In the clinical transplant setting, the primary purpose of a serum antibody analysis is the identification of acceptable HLA mismatches for potential donors. Although this study was done with post-pregnancy sera from healthy women, the data can be used to illustrate the determination of mismatch acceptability of DRB alleles. Table 8 shows for three sera the results with SAB alleles corresponding to serological DR antigens. Determinations of mismatch acceptability of SAB alleles could be solely based on MFI values although information about the presence of antibody-reactive epitopes on such alleles will provide a scientific explanation.

Identifications of epitope specificities of serum antibodies will permit predictions about mismatch acceptability of non-SAB alleles outside the Luminex panel. Serum #22 had antibodies specific for the 67LQ+s60Y pair and for DR1, DR4 and DR52 antigens we could readily predict which non-SAB alleles can be considered acceptable mismatches and which ones are not. Acceptable non-SAB allele mismatches were not found in the DRB1*01:01-10 group (nor 11-20, not shown) but were identified four times in the DRB1*04:06-20 group.

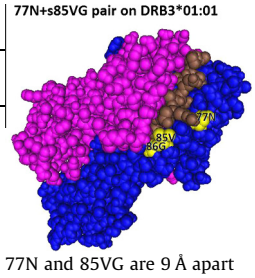
The 70D-epitope specificity of serum #223 led to a DR1 and DR4 mismatch acceptability pattern that was opposite to that for serum #22. Antibody specificities to the other epitopes $142M_3$, 108T and 28H did affect the mismatch acceptability of DR1 and DR4 alleles but this information is of course important for the identification of other DRB alleles (data not shown).

The 31FYY-epitope specificity of serum #178 was applied to determine mismatch acceptability of DR13 alleles; this serum reacted with DRB1*13:03 but not with DRB1*13:01 which was also a self-allele for the antibody-producer. Eleven non-SAB DR13 alleles are acceptable mismatches and seven are unacceptable mismatches (Table 8).

Serum #178 had also $51R_2$-specific antibodies which reacted with DRB3*02:02 but not with DRB3*01:01 and DRB3*03:01. Serum #22 had a different antibody specificity 67LQ+s60Y but showed the same reactivity pattern with these three DR52 alleles. For both sera, DRB3*02:02 is an unacceptable mismatch. The vast majority of DRB3*02 alleles (except DRB3*02:09) are unacceptable mismatches but there was a slight difference between these two sera: DRB3*02:11 is acceptable for serum #22 and DRB3*02:10 is acceptable for serum #178. DRB3*01:07 was unacceptable for both sera.

Table 7
Serum #46 has antibodies specific for the antibody-verified 96HK and 51R2 and three epitopes defined by 47F, 70DA and 77 N+s85VG, respectively.

Serum #46 Antibody producer: DRB1*04:05, *09:01; DRB4*01:01,-							
DRB allele		MFI	Ep1 VerEp	Ep2 Eplet	Ep3 Eplet	Ep4 VerEp	Ep5 Eplet pair
DRB1*13:01	Immunizer	15,562	96HK	47F	70DA		
DRB1*13:03		15,051	96HK		70DA		
DRB1*11:01		15,022	96HK	47F	70DA		
DRB1*11:04		15,472	96HK	47F	70DA		
DRB1*03:01		14,712	96HK	47F			
DRB1*03:02		11,613	96HK				77N+s85VG
DRB1*14:01		13,768	96HK				
DRB1*08:01		13,788	96HK		70DA		
DRB1*12:01		16,379	96HK	47F	70DA		
DRB1*12:02		16,188	96HK	47F	70DA		
DRB1*15:01		7337		47F			
DRB1*15:02		5499		47F			
DRB1*15:03		3768		47F			
DRB1*01:03		14,704			70DA		
DRB1*01:01		16					
DRB1*01:02		5					
DRB1*04:02		15,680			70DA		
DRB1*04:01		1					
DRB1*04:03		1					
DRB1*04:04		1					
DRB1*04:05	Self	23					
DRB1*16:02		13,765			70DA		
DRB1*16:01		12,586			70DA		
DRB5*01:01		11,759			70DA		
DRB1*07:01*		1183*					
DRB3*02:02	Immunizer	10164				$51R_2$	77N+s85VG
DRB3*01:01		5133					77N+s85VG
DRB3*03:01		1					
Negative alleles ($N = 13$)		9 ± 13					
Positive control		7979					



77N and 85VG are 9 Å apart

* The weak reactivity with DRB1*07:01 might reflect the presence of 25QR3-specific antibodies induced during a previous pregnancy.

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Table 8
Three serum examples with epitope specificities that distinguish for selected DR antigens which SAB and non-SAB alleles are acceptable or unacceptable mismatches.

Serum	Epitopes	Mismatched antigen		Acceptable alleles	Unacceptable alleles
#22	67LQ+s60Y	DR1	SAB	DRB1*01:03	DRB1*01:01/02
			Non-SAB		DRB1*01:04/05/06/07/08/09/10
		DR4	SAB	DRB1*04:02	DRB1*04:01/03/04/05
			Non-SAB	DRB1*04:12/14/15/18	DRB1*04:06/07/08/09/10/11/13/16/17/19/20
		DR52	SAB	DRB3*01:01 *03:01	DRB3*02:02
			Non-SAB	DRB3*01:02/03/04 *02:09/11 *03:02/03	DRB3*01:07 *02:01/03/04/05/06/07/08/10/12
#223	142M$_3$, 108T, 28H, 70D	DR1	SAB	DRB1*01:01/02	DRB1*01:03
			Non-SAB	DRB1*01:04/05/06/07/08/09/10	
		DR4	SAB	DRB1*04:01/03/04/05	DRB1*04:02
			Non-SAB	DRB1*04:06/07/08/09/10/11/13/16/17/19/20	DRB1*04:12/14/15/18
#178	51R$_2$, 57DE, 31FYY	DR13	SAB	DRB1*13:01(self)	DRB1*13:03
			Non-SAB	DRB1*13:02/05/06/08/09/10/15/16/18/19/20	DRB1*13:04/07/11/12/13/14/17
		DR52	SAB	DRB3*01:01 *03:01	DRB3*02:02
			Non-SAB	DRB3*01:02/03/04 *02:09/10 *03:02/03	DRB3*01:07 *02:01/03/04/05/06/07/08/11/12

4. Discussion

HLAMatchmaker-based analyses of post-pregnancy sera offer outstanding opportunities to identify HLA epitopes recognized by antibodies. The International Registry of HLA Epitopes http://www.epregistry.com.br has databases for structurally defined HLA-ABC, -DRDQDP and -MICA epitopes which can be divided into two groups: (1) antibody-verified epitopes and (2) eplets that in theory might predict epitopes but which have not been experimentally verified with informative antibodies. The major goal of the Registry is to continually update the repertoires of antibody-verified HLA epitopes and this can only be accomplished by analyzing new antibody reactivity patterns with informative HLA allele panels. The availability of complete epitope repertoires will be useful for the determination of mismatch acceptability and permissibility of potential donors in the clinical transplant setting.

This report describes the reactivity patterns of DRB antibodies with a single allele panel on a Luminex platform. We selected six post-pregnancy sera that reacted differently with groups of alleles within the serologically defined DR1, DR4, DR13 and DR52 antigens. Amino acid differences between alleles in each group were expected to provide information about the structure of DRB epitopes specifically recognized by antibodies.

Our data suggest the identification of eight newly antibody-verified DRB epitopes and three of them are defined by eplet pairs. The DRB1*01:01-induced 67LQ+s60Y (Table 2) and 67LQ+s73AT (Table 3) are structurally similar because both have the 67L and 70Q residues but the eplets are paired with different self-configurations. The third pair is the DRB3*02:02-induced 77N +s85VG which is shared with the reactive DRB3*01:01. This epitope is structurally similar to the antibody-verified 77N reported in the Registry except that it requires 85VG as a second contact site about 9 Å away.

Five newly antibody-verified DRB epitopes correspond to single eplets including 13FE, 47F, 70D and 70DA. Serum #384 and #178 have antibodies specific for epitopes defined by the 31FY+37Y pair but since all residues are within a 3.5 Å radius they comprise one eplet annotated as 31FYY. The specific reactivity of serum #223 with 28H was considered insufficient evidence for a new antibody-verified because the informative 28H-carrying DR9 alleles had very low MFI values.

Serum #384 (Table 5) and serum #178 (Table 6) had DRB1*11:04-induced specificity patterns that corresponded with the antibody-verified 31FYY epitope, but there some differences between their reactivity patterns. The reactivity of #384 with 31FY+37F-carrying DRB1*07:01 suggested a serological cross-reactivity between 31FYY and 31FY+37F. High reactivity of #178 with 31FYY-carrying alleles was associated with the sharing of certain residues in nearby sequence positions 10, 11, 12 and 33 with the immunizing DRB1*11:04 allele. Less reactive alleles have different residues than the immunizing allele and the more reactive alleles. This reflects a cross-reactivity at the eplet level whereby certain nearby residue differences affect the expression of the corresponding epitope.

As another example, the comparable reactivity pattern of 67LQ +s73AT-carrying and 67LR+s73AT-carrying alleles with #93 (Table 3) seems to reflect a strong serological cross-reactivity between 67LQ and 67LR eplets. On the other hand, 67LQ+s60Y-carrying alleles reacted much stronger with #22 than 67LR+s60Y-carrying alleles (Table 2) thereby suggesting that 67LR is considerably less cross-reactive with 67LQ for this antibody.

In HLA serology, the term cross-reactivity refers to groups of antigens or alleles that share epitopes, the so-called cross-reacting groups or CREGs [9–11]. Cross-reactivity at the epitope level deserves a different consideration if two or more structurally similar eplets or eplet pairs with residue differences that have no effect or only modestly affect the reactivity with specific antibody. This means that certain residue substitutions are allowable for the expression of the epitope and this reflects the so-called Landsteiner type of cross-reactivity. Classic experiments in 1935 were conducted with antibodies generated to synthetic haptens conjugated to protein carriers [12]. These antibodies were hapten-specific but they reacted also with different affinities to chemically related haptens. This cross-reactivity of immunological specificity applies also to antigenic determinants on proteins [13] and relates to a structural complementarity which permits interactions between epitope and antibody involving hydrogen bonds, ionic interactions, van der Waals forces and hydrophobic interactions. Kosmoliaptsis addresses these physiochemical concepts for HLA epitopes [14,15].

Serum #93 revealed antibody specificity to an epitope defined by a pair of self-eplets: s67LQ+s73AT (Table 3). Recent studies have shown that certain antibodies are specific for class I epitopes defined by pairs of self-eplets [16–19]. These findings are consistent with the nonself-self paradigm of HLA epitope immunogenicity which considers the concept that HLA antibodies originate from B-lymphocytes with low-affinity immunoglobulin B-cell receptors for self-HLA epitopes [20]. Such receptors can have a productive interaction with a non-self eplet whereby the remainder of the structural epitope on the immunizing antigen is essentially self for the antibody producer.

Epitope specificity analysis of serum HLA antibodies permits a better determination of mismatch acceptability for sensitized patients. Any allele with an epitope recognized by patient's antibodies should be considered as an unacceptable mismatch. This

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report illustrates that serological DR types cannot readily identify acceptable mismatches; these findings are consistent with the viewpoint that matching should done at the allele level rather than at the antigen level [1].

Disclosures

This study was approved by the local ethics committee and written informed consent was obtained from women enrolled in the study. Dr Schaub is supported by the Swiss National Foundation (Grant 32473B_125482/1) and the Nora van Meeuwen-Hafliger foundation. The authors of this manuscript have no conflicts of interest to disclose.

References

[1] R.J. Duquesnoy et al., Personal viewpoint: should HLA mismatch acceptability for sensitized transplant candidates be determined at the high-resolution rather than the antigen level?, Am J. Transplant. 20 (2015) 1–6.
[2] R.J. Duquesnoy et al., 16th IHIW: a website for the antibody-defined HLA epitope Registry, Int. J. Immunogenet. 40 (2013) 54–59.
[3] R.J. Duquesnoy et al., First report on the antibody verification of MICA epitopes recorded in the HLA epitope registry, Int. J. Immunogenet. 41 (2014) 370–377.
[4] R.J. Duquesnoy et al., First report on the antibody verification of HLA-ABC epitopes recorded in the website-based HLA epitope registry, Tissue Antigens 83 (6) (2014) 391–400.
[5] R.J. Duquesnoy et al., First report on the antibody verification of HLA-DR, HLA-DQ and HLA-DP epitopes recorded in the HLA epitope registry, Hum. Immunol. 75 (2014) 1097–1103.
[6] G. Honger et al., Frequency and determinants of pregnancy-induced child-specific sensitization, Am. J. Transplant. 13 (3) (2013) 746–753.
[7] W. Klitz et al., New HLA haplotype frequency reference standards: high-resolution and large sample typing of HLA DR-DQ haplotypes in a sample of European Americans, Tissue Antigens 62 (4) (2003) 296–307.
[8] L. Gragert et al., Six-locus high resolution HLA haplotype frequencies derived from mixed-resolution DNA typing fro the entire US donor registry, Hum. Immunol. 74 (2013) 1313–1320.
[9] G.E. Rodey, T.C. Fuller, Public epitopes and the antigenic structure of the HLA molecules, Crit. Rev. Immunol. 7 (3) (1987) 229–267.
[10] A.A. Fuller et al., Epitope map of the HLA-B7 CREG using affinity-purified human alloantibody probes, Hum. Immunol. 28 (3) (1990) 306–325.
[11] A.A. Fuller et al., Topographic map of the HLA-A2 CREG epitopes using human alloantibody probes, Hum. Immunol. 28 (3) (1990) 284–305.
[12] K. Landsteiner, J. Jacobs, Studies on the sensitization of animals to simple chemical compounds, J. Exp. Med. 62 (1936) 625–639.
[13] K. Landsteiner, The Specificity of Serological Reactions (Revised Edition), Dover Publications Inc., New York, NY, 1962.
[14] V. Kosmoliaptsis et al., High-resolution, three-dimensional modeling of human leukocyte antigen class I structure and surface electrostatic potential reveals the molecular basis for alloantibody binding epitopes, Hum. Immunol. 72 (11) (2011) 1049–1059.
[15] D.H. Mallon et al., Three-dimensional structural modelling and calculation of electrostatic potentials of HLA Bw4 and Bw6 epitopes to explain the molecular basis for alloantibody binding: toward predicting HLA antigenicity and immunogenicity, Transplantation 99 (2) (2015) 385–390.
[16] M. Marrari et al., Brief report: Why did two patients who type for HLA-B13 have antibodies that react with all Bw4 antigens except HLA-B13?, Transplant Immunol 25 (2011) 217–220.
[17] R.J. Duquesnoy, M. Marrari, A. Mulder, Usefulness of the nonself-self algorithm of HLA epitope immunogenicity in the specificity analysis of monospecific antibodies induced during pregnancy, Front. Immunol. 6 (2015). Article 160.
[18] A. Hahn et al., HLA-A2 reactive antibodies in a patient who types as HLA-A2: the importance of high 3 resolution typing and epitope-based antibody analysis, Transplant Immunol. 32 (2015) 141–143.
[19] M. Resse et al., Antibody-reactive class I epitopes defined by pairs of mismatched eplets and self-eplets, Tissue Antigens 86 (2015) 368–372.
[20] R.J. Duquesnoy, The antibody response to an HLA mismatch: a model for nonself-self discrimination in relation to HLA epitope immunogenicity, Int. J. Immunogenet. 39 (2011) 1–9.