INTERNATIONAL JOURNAL OF
IMMUNOGENETICS

doi: 10.1111/iji.12280

Detection of newly antibody-defined epitopes on HLA class I alleles reacting with antibodies induced during pregnancy

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Summary

The determination of HLA mismatch acceptability at the epitope level can be best performed with epitopes that have been verified experimentally with informative antibodies. The website-based International Registry of HLA Epitopes (http://www.epregistry.com.br) has a list of 81 antibody-verified HLA-ABC epitopes but more epitopes need to be added. Pregnancy offers an attractive model to study antibody responses to mismatched HLA epitopes which can be readily determined from the HLA types of child and mother. This report describes a HLAMatchmaker-based analysis of 16 postpregnancy sera tested in single HLA-ABC allele binding assays. Most sera reacted with alleles carrying epitopes that have been antibody-verified, and this study focused on the reactivity of additional alleles that share other epitopes corresponding to eplets and other amino acid residue configurations. This analysis led in the identification of 16 newly antibody-defined epitopes, seven are equivalent to eplets and nine correspond to combinations of eplets in combination with other nearby residue configurations. These epitopes will be added to the repertoire of antibody-verified epitopes in the HLA Epitope Registry.

Introduction

There is now increasing support of the concept that mismatch acceptability for sensitized transplant patients should be determined at the HLA epitope level and involve HLA typing at high resolution rather than antigen levels (Terasaki *et al.*, 1992; Duquesnoy *et al.*, 2004, 2015; Cai *et al.*, 2008; Wiebe & Nickerson, 2014). HLAMatchmaker is an algorithm designed to predict HLA epitopes by molecular structural modelling and amino acid sequence comparisons between HLA alleles (Duquesnoy, 2006). It considers each HLA 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 eplets encoded by the various HLA loci (Duquesnoy *et al.*, 2013). An important question is which eplets correspond to actual epitopes specifically recognized by HLA antibodies.

During the past two decades, many investigators have reported unique HLA epitopes defined by antibodies and three recent publications describe antibody-verified (AbVer) HLA-ABC, -DRDQDP and MICA epitopes recorded so far in the HLA Epitope Registry (Duquesnoy, 2014a,b). All of them correspond to eplets and there are two patterns. First, a specific antibody reacts with all alleles carrying a given eplet, whereas the remaining alleles in the panel are nonreactive. In these cases, an eplet describes the epitope specifically recognized by antibody. Second, an epitope is defined by the combination of an eplet and another polymorphic residue configuration (eplet) uniquely shared between all antibody-reactive alleles. Such epitopes are described by so-called eplet pairs. For instance, the 82LR eplet represents an AbVer epitope shared between Bw4 specific alleles, whereas the 82LR+145R epitope is expressed on all Bw4 alleles except B*13:01 and B*13:02 which have 82LR+145L (Marrari *et al.*, 2011). There are several other variations of Bw4 including 82LR+138T on HLA-B and 82LR+138M on HLA-A (Duquesnoy, 2014a,b).

As of 1 January 2016, the HLA Epitope Registry has recorded 81 AbVer HLA-ABC epitopes, 62 of them correspond to eplets and 19 are defined by eplet pairs. This list is incomplete and more AbVer epitopes need to be added.

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Received 9 February 2016; revised 10 May 2016; accepted 19 May 2016

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Pregnancy offers an attractive model to study antibody responses to mismatched HLA epitopes which can be readily determined from the HLA types of child and mother. A previous study of postpartum serum reactivity with single-allele beads (SAB) has shown HLA class I antibodies in about 30% of the pregnancy cases and the frequencies of antibody responses correlate with the number of mismatched eplets on the paternal alleles of the child (Honger *et al.*, 2013).

HLAMatchmaker is a useful tool to determine how the positive reactions of alleles in an HLA-typed panel correlate with the presence of specific epitopes recognized by HLA antibodies (Duquesnoy, 2014a,b). Such epitopes could belong to the list of AbVer epitopes already recorded in the HLA Epitope Registry, or they could correspond to other eplets not yet considered as AbVer. This report describes an HLAMatchmaker analysis of 16 postpregnancy sera with child-specific antibodies with reactivity patterns that correspond to new epitopes. Such epitopes are candidates to be added to the repertoire of AbVer epitopes in the HLA Epitope Registry.

Materials and methods

Populations and HLA typing and antibody testing methods

This study was carried out on sera from women giving full-term live birth at the University Hospital Basel between September 2009 and April 2011. A blood sample was drawn from the mother between day 1 and 4 after delivery for high-resolution HLA-ABC typing and antibody testing. Cord blood of the child was obtained immediately after delivery for high-resolution HLA typing.

HLA antibody testing was made with single HLA antigen beads (SAB) for class I (iBeads, lot 1; One Lambda, ThermoFisher, Los Angeles, CA, USA) and normalized mean fluorescence intensity (MFI) values were determined for each bead as previously described. Cut-off values between positive and negative reactions were generally based on MFI>300 values as previously reported (Honger *et al.*, 2013).

HLAMatchmaker analysis

HLAMatchmaker is a computer program that predicts for immunizing alleles which structurally defined epitopes are mismatched for the antibody producer and which can be used to analyse epitope specificities of antibodies reacting with HLA-typed panels (Duquesnoy, 2014a,b). This analysis of postpregnancy sera consisted of two steps. First, we determined which mismatched AbVer epitopes are shared between the child's immunizing paternal alleles and the reactive alleles of the SAB panel. Moreover, we also considered differences of MFI values between reactive alleles to assess the relative strength of antibodies reacting with various epitopes. The second step was the analysis of reactive alleles in the SAB panel that might carry other epitopes shared with the immunizing allele but which have not been reported in the HLA Epitope Registry as being AbVer. The successful outcome of this analysis depends on the availability of 'informative' alleles that uniquely share a newly identified epitope with the immunizing allele but lack the child-specific AbVer epitopes recognized by antibodies in the pregnancy serum. Such putative epitopes might be considered candidates for being recorded as AbVer in the HLA Epitope Registry.

There are two categories of newly identified epitopes. First, they are equivalent to eplets if all reactive alleles share the eplet with the immunizing allele. This means that an eplet recorded in the HLA Epitope Registry could be upgraded as AbVer. Second, certain of eplet-carrying alleles react with antibody and they require the presence of additional amino acids which according to the structural epitope concept (Duquesnoy, 2014a,b) cannot be more than 15 Ångstroms away from the eplet. This concept is based on the fact that antibodies have six complementarity determining regions (CDRs) that bind to amino acid residues of a structural protein epitope including a centrally located CDR which plays a dominant role in antibody specificity. Cn3D molecular modelling (Hogue, 1997) can identify such residues, and relevant polymorphic residue configurations must be present on the immunizing allele and all the eplet-carrying reactive alleles, whereas nonreactive, eplet-carrying alleles have different configurations. Previous studies have demonstrated that epitopes defined by eplet pairs consist of non-self-eplets together with self-configurations also found on at least one allele of the antibody producer (Marrari *et al.*, 2010). Our analysis in this report also addresses this issue.

Selection of postpregnancy sera

Our studies addressed the reactivity of child-specific antibodies after one or two pregnancies. As these were healthy women, it is presumed that pregnancy was the only sensitization event. One must raise the question whether the antibody reactivity after a second pregnancy is always specific for the second child and how many sera might have antibodies induced to a different haplotype during the first pregnancy. With the same father, one can calculate a 50% chance of different paternal haplotypes between first and second pregnancies (for the latter, there was no typing information for the first child). Previous studies have shown a 30% incidence of antibodies after a first pregnancy (Honger *et al.*, 2013), so the overall chance of antibodies against the other haplotype is only 15%. Moreover, it is also possible that the time interval until the second pregnancy might lead to a reduction or even the disappearance of antibodies induced during the first pregnancy. Although interfering antibodies with unrelated specificities seemed unlikely, we considered this potential problem in our analysis.

Results

Serum reactivity patterns of sera with child-reactive HLA antibodies

Table 1 and Table 2 describe six first (1st P) and ten second (2nd P) well-reacting postpregnancy sera. They have columns for the HLA types of the antibody producers and the paternal alleles of the child along with their MFI values. These sera were selected for analysis if at least one paternal allele had a MFI>2000. Some sera such as 1st P#228 reacted with all paternal alleles and others reacted with two (e.g. 1st P#24) or one allele (e.g. 1st P#233). Certain paternal alleles were not in the SAB panel; in such cases, the MFI of a corresponding allele was recorded. For example, C*07:01 (1st P#49) was a child allele absent from the SAB panel and the MFI value for C*07:02 was used; it is 02=1402. Altogether, there were 11/17 (65%) first and 17/26 (65%) second pregnancy mismatched paternal alleles with >2000 MFI values.

These tables have also columns for the AbVer child-specific epitopes identified with HLAMatchmaker. For instance, 1st P#24 reacted with 62GE and 144TKH presented by the immunizing A*02:01 but no AbVer epitopes were identified on the immunizing B*44:02 (MFI = 6742); child's C*05:01 (MFI = 376) was considered nonreactive. Altogether, AbVer epitopes were identified 40 times on the 28 reactive mismatched alleles. In 36 cases, such epitopes corresponded to single eplets and four eplet pairs were found.

The major goal of this study was to determine whether the reactivity of other alleles in the panel could be explained with the presence of additional child-specific epitopes. The far right columns of Tables 1 and 2 describe these epitopes along with MFI values and the numbers of reactive informative alleles. For instance, 1st P#24 reacted with a new epitope 79GT presented by the immunizing A*02:01 and another new epitope 163L+167S/G presented by the immunizing B*44:02. The next section has brief descriptions how these new epitopes were identified.

Details of our analysis of the reactivity patterns of first and second postpregnancy sera including MFI values of informative alleles can be found in reports of newly antibody-verified epitopes on the HLA Epitope Registry website.

New antibody-defined epitopes presented by child HLA-A alleles

79GT epitope on all HLA-A alleles except A23, A24, A25 and A32

1st P#24 had antibodies that had been induced by A*02:01 and reacted well with alleles carrying the AbVer 144TKH and 62GE epitopes. This serum reacted also with all other HLA-A alleles in the panel except A*23:01, A*24:03, A*25:01 and the self-alleles A*24:02 and A*32:01. This reactivity corresponds to the presence of the 79GT eplet. This means that all 79GT-carrying alleles are reactive; there were 20 informative alleles (all of them lack 144TKH and 62GE) and their mean MFI = 1937 ± 1155.

2nd P#217 had A*03:01-induced antibodies with almost the same HLA-A allele reactivity pattern as 1st P#24. Two alleles were highly reactive: A*03:01 (MFI = 12869) which has a unique AbVer 161D and A*66:02 (MFI = 10509) which shares 163EW+s66I/N

Table 1. Reactivities of first pregnancy sera with alleles carrying antibody-verified and newly defined child-specific epitopes

Serum 1st Pregnancy	HLA Type of Antibody Producer	Child Allele	MFI	Child-Specific Antibody-Verified Epitopes	New Child-Specific Epitopes (Mean ± SD MFI, Nr of Informative Alleles)
1stP#24	A*24:02 A*32:01	A*02:01	7609	62GE,144TKH	79GT (1937 ± 1156, *N* = 20))
	B*07:02 B*41:02	B*44:02	6742	None identified	163L+167S/G (7920 ± 2484, *N* = 5)
	C*07:02 C*17:03	C*05:01	376	Non-reactive	
1stP#49	A*02:01 A*02:01	A*01:01	11 818	90D, 76ANT, 144KR, 166DG	138MI (8110 ± 1741, *N* = 8)
	B*15:01 B*51:01	B*58:01	6305	65RNA+s80I	
	C*01:02 C*03:03	C*07:01	02=1402	90D	
1stP#186	A*11:01 A*24:02	A*03:02	01=286	Non-reactive	
	B*07:02 B*39:06	B*27:05	8953	71ATD	
	C*07:02 C*07:02	C*01:02	7245	None identified	$6K_2$ (7245, *N* = 1)
1stP#228	A*01:01 A*24:02	A*02:01	10 395	144TKH	166EW+s65R+s158A (4633 ± 1240, *N* = 2), 207S (4483, *N* = 1)
	B*08:01 B*40:02	B*15:01	9061	44RMA, 163LW	131S (11102 ± 2059, *N* = 17)
	C*02:02 C*07:01	C*03:04	6174	173K, 163LW	
1stP#233	A*02:01 A*23:01	A*02:01	39	Match	
	B*15:18 B*51:01	B*40:02	9647	41T	163EW+s66I/ *N* (8306 ± 1956, *N* = 7)
	C*07:04 C*15:02	C*02:02	430	Non-reactive	
1stP#254	A*01:01 A*03:01	A*31:01	2	Non-reactive	
	B*40:02 B*57:01	B*27:05	11	Non-reactive	
	C*02:02 C*06:02	C*01:02	3025	None identified	76VRN+152sE/T/V (2168 ± 709, *N* = 8)

Table 2. Reactivities of second pregnancy sera with alleles carrying antibody-verified and newly defined child-specific epitopes

Serum 2nd pregnancy	HLA Type of antibody producer	Child allele	MFI	Child-Specific antibody-verified epitopes	New Child-specific Epitopes (Mean ± SD MFI, Nr of Informative Alleles)
2ndP#61	A*24:02 A*24:02	A*02:01	13 091	62GE, 144TKH	79GT (MFI=5827 ± 2823, *N* = 7), 207S/194AV (MFI=5333 ± 472, *N* = 2)
	B*15:01 B*55:01	B*18:01	8095	None identified	170RH+s62RN (2611 ± 2033, *N* = 6)
	C*03:03 C*03:03	C*07:01	02 = 29	Non-reactive	
2ndP#93	A*02:01 A*03:01	A*03:01	37	Match	
	B*37:01 B*44:02	B*35:01	13 824	80N, 163LW	113H (MFI=2507 ± 1293, *N* = 5)
	C*05:01 C*06:02	C*04:01	659	None identified	
2ndP#95	A*01:01 A*03:01	A*29:02	2788	None identified	62LQ+s163T (2634 ± 218, *N* = 2)
	B*35:01 B*44:02	B*44:03	1	Non-reactive	
	C*04:01 C*05:01	C*16:01	1	Non-reactive	
2ndP#102	A*01:01 A*24:02	A*03:01	13 090	161D, 76VDT+s144K	66NV (1995 ± 1017, *N* = 8)
	B*07:02 B*39:06	B*07:02	1	Match	
	C*07:02 C*07:02	C*07:02	1	Match	
2ndP#124	A*01:01 A*30:01	A*11:01	52	Non-reactive	
	B*07:02 B*08:01	B*35:01	6534	44RT	131S (4138 ± 1634, *N* = 31)
	C*07:01 C*07:02	C*04:01	1	Non-reactive	
2ndP#164	A*11:01 A*24:02	A*11:01	6	Non-reactive	
	B*15:18 B*51:01	B*07:02	10 843	65QIA, 69AA, 180E	163EW+s170R (MFI=12099 ± 2734, *N* = 7)
	C*07:04	C*07:04	02=35	Match	
2ndP#217	A*24:02 A*24:02	A*03:01	12 869	161D	79GT (3008 ± 1598, *N* = 17)
	B*15:01 B*57:01	B*07:02	12 482	65QIA, 180E	163EW+s66I/N (9708 ± 3309, *N* = 6)
	C*03:03 C*06:02	C*07:02	24	Non-reactive	
2ndP#348	A*02:01 A*68:01	A*01:01	10 023	144KR, 166DG, 62QE+s56R	
	B*44:02 B*50:01	B*57:01	9163	44RMA, 69AA, 80I	113H (10142 ± 2097, *N* = 13)
	C*06:02 C*07:04	C*06:02	1	Match	
2ndP#364	A*02:01 A*02:01	A*11:01	9136	144KR, 163R	138MI (9060 ± 2469, *N* = 3), 66NV (7549 ± 4138, *N* = 3)
	B*44:02 B*49:01	B*27:05	11 790	65QIA	163EW+s170R (6029 ± 3120, *N* = 7)
	C*05:01 C*07:01	C*02:02	3632	None identified	
2ndP#430	A*02:01 A*02:01	A*24:02	4270	82LR	
	B*15:01 B*35:01	B*51:01	11 486	82LR, 163T+s73TE	170RH+s103V (6433 ± 1694, *N* = 5)
	C*03:03 C*04:01	C*15:02	8994	None identified	80K+s14R (4842 ± 1968, *N* = 5)

with the immunizing B*07:02. All 25 informative 79GT-carrying alleles were reactive (MFI = 3008 ± 1598).

2nd P#61 had A*02:01-induced antibodies that reacted with all HLA-A alleles except A*23:01, A*24:03 and the antibody producer's A*24:02. Besides the high reactivity with alleles carrying the AbVer 144TKH and 62GE, this serum reacted also well with 79GT on 7 informative alleles (MFI = 5827 ± 2823). Reactivity with A*25:01 and A*32:01 reflected antibody responses to 193AV or 207S.

138MI epitope on all HLA-A alleles except A2, A68 and A69

1st P#49 came from a woman who was homozygous for A*02:01 and had A*01:01-induced antibodies that reacted with alleles carrying the AbVer 166DG (B*15:12 with MFI = 13108 was informative) and the AbVer 90D (B*73:01 with MFI = 11822 was informative). All HLA-A alleles except the A2, A68 and A69 alleles were reactive and they shared the 138MI eplet. A30, A31, A33, A74 and A*66:02 (MFI = 8110 ± 1741) were informative for 138MI-specific antibodies.

2nd P#364 came also from an A*02:01 homozygous woman who had A*11:01-induced antibodies that reacted with all HLA-A alleles except A2. The reactive alleles shared the AbVer 144KR and 163R epitopes. Two new epitopes were identified on the remaining reactive HLA alleles. First, A*23:01, A*34:01 and A*34:02 (MFI = 9060 ± 2469) were informative for 138MI and second, 66NV.

*166EW+s65R+s158A epitope on all HLA-A alleles except A1, A23, A*24:02 and A80 and also on B*15:16, B57 and B58*

1st P#228 had A*02:01-induced antibodies that reacted with all HLA-A alleles except A*23:01 and the antibody producer's A*01:01 and A*24:02. The reactive HLA-A alleles carried the AbVer 144TKH epitope, and as shown below, the serum had also antibody reactivity HLA-A alleles with a 207S-defined epitope. As the negative HLA-A alleles carried the AbVer 166DG epitope, we postulated the presence of antibodies specific for the alternative 166EW epitope in the same sequence position. As many 166EW-carrying HLA-B and HLA-C alleles were nonreactive, we concluded that the

structural description of this epitope must include other residues within a 15 Ångstrom radius. The combination between 166EW and self-residues 65R and 158A (which are 7 and 10 Ångstroms away from 166EW) can fully define the epitope on 1st P#228-reactive HLA-A alleles. This epitope is called 166EW+s65R+s158A, and both A30 alleles (MFI = 4633 ± 1240) were informative. Two 163EW-carrying HLA-A alleles were weakly reactive: A*36:01 (MFI = 814) has 158V instead of 158A, whereas A*24:03 (MFI = 1325) has 65G instead of 65R.

There were four 166EW+s65R+s158A-carrying HLA-B alleles namely B*15:16, B*57:01, B*57:03 and B*58:01, and they were highly reactive (MFI = 12140 ± 620). These alleles were not considered informative because they carried also AbVer 163LW and 44RMA and a newly defined 131S epitope apparently recognized by child's B*15:01-induced antibodies in 1st P#228.

*66NV epitope on A3, A11, A25, A26, A29, A30, A31, A32, A*34:02, A43, A66, A68, A69, A74 and A80 alleles*

2nd P#364 had A*11:01-induced antibodies reacting with all HLA-A alleles except A2 including the self-allele. The reactive alleles shared the AbVer 144KR and 163R epitopes. Two new epitopes were identified on the remaining reactive HLA alleles: 138MI and 66NV. A*68:01, A*68:02 and A*69:01 (MFI = 7549 ± 4138) were informative for 66NV.

2nd P#102 had A*03:01-induced antibodies that reacted with all HLA-A alleles except A*01:01 (self) A*23:01, A*24:02 (self) and A*36:01. The immunizing A*03:01 (MFI = 13090) has a unique AbVer 161D. This serum had also antibodies reacting with all 66NV-carrying alleles, and A*25:01, A*26:01, A*29:01, A*29:02, A*30:02, A*32:01, A*43:01, A*69:01 and A*80:01 (MFI = 1995 ± 1017) were informative.

207S on A2, A25, A26, A29, A31, A32, A33, A34, A43, A66, A68, A69, A74 and A80 alleles

1st P#228 had A*02:01-induced antibodies that reacted with HLA-A alleles expressing the AbVer 144TKH and 76VDT+s144K and also as described above the newly defined 166EW+s65R+s158A epitope. This serum had additional antibody reactivity with a 207S-defined epitope on HLA-A. Most 207S-carrying alleles have also 166EW+s65R+s158A, but there was one well-reacting allele A*80:01 (MFI = 4483) which is informative for 207S.

2nd P#61 had A*02:01-induced antibodies that reacted with all HLA-A alleles except A*23:01, A*24:03 and the antibody producer's A*24:02. Besides the high reactivity with alleles carrying the AbVer 144TKH and 62GE, this serum reacted also well with 79GT and with 207S-carrying alleles and A*25:01 and A*32:01 (MFI = 5333 ± 472) were informative.

*62LQ+s163T epitope on A*29:01 and A*29:02*

2nd P#95 reacted only with A*29:01 and the immunizing A*29:02 (MFI = 2634 ± 218). These alleles share a unique 62LQ with A*43:01 which was however nonreactive (MFI = 78). This allele has a nearby residue difference position 7.5 Ångstroms away: 163R versus 163T a self-residue on A*03:01 of the antibody producer. This suggests the presence of a new epitope pair defined by 62LQ+s163T.

New antibody-defined epitopes presented by child HLA-B alleles

131S epitope on B13, B14, B15, B18, B27, B35, B37, B38, B39, B44, B45, B46, B47, B49, B50, B51, B52, B53, B54, B55, B56, B57, B58, B59, B67, B78, B82 alleles

1st P#228 had B*15:01-induced antibodies that reacted strongly only with 131S-carrying HLA-B alleles. Although several of them carry also the AbVer 44RMA and 163LW epitopes also present on the child's B*15:01, we identified 17 informative 131S-carrying alleles (MFI = 11102 ± 2059) with only 131S.

2nd P#124 had B*35:01-induced antibodies with broad reactivity with HLA-B alleles, and it did not react with A*11:01 and C*04:01 of the child. The reactive HLA-B alleles shared the AbVer 44RT with the child but this reactivity pattern included also all 131S-carrying alleles and 31 informative alleles had an MFI = 4138 ± 1634.

*163LS/G epitope on B44, B45 and B82 alleles and B*15:12*

1st P#24 had B*44:02-induced antibodies that reacted well with not only B*44:03, B*45:01 and B*82:01 (which share the 166ES eplet) but also equally well with B*15:12 which lacks 166ES. A structural and residue analysis showed that B*44:02, B*44:03, B*45:01 and B*82:01 have an epitope defined by the unique combination of 163L and 167S; these residues are 3 Ångstroms apart thereby suggesting a new eplet 163LS. The reactive B*15:12 has a unique 163L+167G combination. The residue difference in sequence position 167 involves serine replaced by the structurally similar glycine, and this appears to be a permissible substitution which did not affect the reactivity of this B*44:02-induced antibody in #24. Accordingly, the epitope can be defined as 163LS/G.

*163EW+s66I/N epitope on A*66:02, B7, B13, B27, B40, B47, B48, B73 and B81 alleles*

1st P#233 had B*40:02-induced antibodies specific for the AbVer 41T. This serum reacted also with 163EW-carrying B7, B27, B47, B48, B73, B81 and A*66:01 alleles (MFI = 8306 ± 1956), whereas the 163EW-carrying C*02:02 (MFI = 430) and C*17:01

(MFI = 67) were considered nonreactive. These findings indicated a new epitope defined by 163EW paired with another configuration. Structural modelling and residue comparisons suggested a role of position 66 (8 Ångstroms away) because all 163EW-carrying HLA-B alleles are reactive and they have the self-residue 66I, whereas 66K is present on the nonreactive HLA-C alleles. It should be noted that 1st P#233 reacts with A*66:02 (MFI = 7976) which is the only 163EW-carrying HLA-A allele in the panel but which has 66N instead of 66I. This presents an example of cross-reactivity whereby the substitution of isoleucine by asparagine does not affect the reactivity with antibodies in 1st P#233. The new epitope can be defined as 163EW+s66I/N.

2nd P#217 had B*07:02-induced antibodies that reacted with the AbVer 65QIA and 180E epitopes. A third reactivity pattern corresponded with the presence of an epitope defined by 163EW+s66I/N, and there were six informative alleles B*13:01, B*13:02, B*40:02, B*40:06 B*47:01 and A*66:02 (MFI = 9708 ± 3309).

*163EW+s170R epitope on A*66:02, B7, B13, B27, B40, B47, B48, B73, B81 and C*02:02 alleles*

2nd P#164 had B*07:02-induced antibodies that reacted with alleles with the AbVer 65QIA, 69AA and 180E epitopes. This serum reacted also with all 163EW-carrying alleles except C*17:01 (MFI = 448). Structural modelling and residue comparisons suggested the influence of self-residue 170R which is only 3.5 Ångstroms away from 163EW; the nonreactive C*17:01 has 170G. This suggests an epitope defined by 163EW+s170R, and B*13:01, B*13:02, B*40:02, B*40:06, B*47:01 and A*66:01 (MFI = 12099 ± 2734) were informative alleles. The 163EW+s170R-carrying C*02:02 (MFI = 2378) was also informative but less reactive; this allele has a nearby 66K residue that appeared to diminish the binding strength with antibody.

2nd P#364 had B*27:05-induced antibodies that were specific for the AbVer 65QIA. The remaining reactivities with HLA-B alleles were virtually identical to those seen with 2nd P#164 and corresponded to the presence of the 163EW+s170R epitope. Seven informative alleles had MFI = 6029 ± 3120, and again, the 163EW+s170R-carrying C*02:02 (MFI = 3632) was less reactive.

*113H on all HLA-B alleles except B14, B*15:02, B*15:13, B27, B37, B44, B45, B47, B49, B50 and B73 but present on C*15:02*

2nd P#93 had B*35:01-induced that reacted well with the AbVer 80N- and 163LW-carrying alleles, and there was a third reactivity pattern that corresponded to the presence of 113H. The B13, B38 and B59 alleles (MFI = 2815 ± 1263) were informative. C*15:02 as the only 113H-carrying HLA-C allele was slightly less reactive (MFI = 1275). These findings suggest the presence of antibodies directed to an epitope defined by 113H which is visible only on the underside of the B*35:01 molecule.

2nd P#348 had B*57:01-induced antibodies that reacted well with alleles with the AbVer 45RMA, 69AA and 80I epitopes. There was another reactivity pattern that corresponded with the presence of 113H, and there were 12 informative HLA-B alleles (MFI = 10 142 ± 2097) plus the 113H-carrying C*15:02 (MFI = 9259) which was the only reactive HLA-C allele.

*170RH+s103V epitope on A*33:01, B14, B18, B*51:01, B52 and B78 alleles*

2nd P#430 had B*51:01-induced antibodies that reacted with the AbVer 82LR. Four reactive 82LR-negative HLA-B alleles B*14:01, B*14:02, B*18:01 and B78:01 (MFI = 6201 ± 2044) share 170RH with the immunizing B*51:01. A*33:01 as the only 170RH-carrying HLA-A allele was reactive (MFI = 5235 versus MFI = 253 of the 170RY-carrying A*33:03). However, the remaining 170RH-carrying B*73:01 (MFI = 253) was considered nonreactive. These findings suggest that this 170RH-defined epitope must include another residue configuration. Molecular modelling suggests the involvement of sequence position 103 which is 9.5 Ångstroms away from 170RH. B*73:01 has 103M, whereas all reactive 170RH-carrying alleles share 103V with the immunizing B*51:01. The latter is a self-residue so this new epitope can be defined as 170RH+s103V.

*170RH+s62RN epitope on B14, B18, B*51:01, B73 and B78 alleles*

2nd P#61 reacted well with the immunizing B*18:01 (MFI = 8095) which has a unique 30G residue and was also reactive with all 170RH-carrying alleles (MFI = 2611 ± 2033) except B*52:01 (MFI = 91). This means that this epitope involves another configuration which turned out to be a self-62RN about 10 Ångstroms from 170RH. In contrast, the nonreactive B*52:01 has 62RE. This suggests an epitope defined by 170RH+s62RN. The weakly reactive B*73:01 (MFI = 671) has as noted above, 103M rather than 103V suggesting an inhibitory effect of 102M.

New antibody-defined epitopes presented by child HLA-C alleles

*$6K_2$ epitope on C*01:02*

1st P#186 had B*27:05-induced antibodies that reacted strongly only with the immunizing allele (MFI = 8953) but not with any other HLA-B allele. B*27:05 has a unique epitope defined by 71ATD. This serum reacted strongly with the immunizing C*01:02 (MFI = 7245) but none of the other HLA-C alleles.

C*01:02 has a distinct eplet $6K_2$ which reflects two possible residues: 6K and 248M.

76VRN+s152E/T/V on C1, C3, C8, C12, C14, B46 and B73 alleles

1st P#254 had only C*01:02-induced antibodies, and they reacted with all 76VRN-carrying alleles (MFI = 2168 ± 709) except C*07:02 (MFI = 92) and C*16:01 (MFI = 210) which share residue 152A which is not on the reactive alleles. The immunizing C*01:02 as well as C*03:02/03/04, C*12:03, C*14:02 and B*46:01 have 152E which is a self-residue about 9 Ångstroms away from 76VRN. Therefore, the epitope can be defined as 76VRN+s152E. We noted that the comparably reacting 76VRN-carrying C*08:01 and B*73:01 have 152T and 152V, respectively. This suggests that these residue substitutions in sequence position 152 do not affect the reactivity with antibody, whereas the presence of 152A seems associated with a lack of binding with 1st P#254. This suggests an epitope which can be described as 76VRN+s152E/T/V whereby E/T/V indicates permissible residue substitutions.

80K+s14R epitope on C2, C5, C6, C15, C17 and C18 alleles

2nd P#430 had C*15:02-induced antibodies that reacted with all 80K-carrying alleles except C*04:01 which has a distinct 14W residue rather than 14R found on all other HLA-C alleles. The latter is a self-residue 13 Ångstroms away from 80K. This suggests an epitope defined by 80K+s14R, and there were five informative alleles (MFI = 4842 ± 1968).

Listing of newly antibody-defined epitopes on reactive alleles

Table 3 shows the list of newly antibody-defined epitopes on alleles reactive with one or more postpregnancy sera together with the alleles in the SAB panel and their frequencies in a National Marrow Donor Panel. Seven epitopes are equivalent to eplets, and nine epitopes are defined by combinations of eplets with other amino acid configurations.

It should be noted that several epitopes have >90% frequencies, and this explains why after sensitization by a single allele, certain sera react with so many alleles and why the HLA types of the antibody producers show homozygosity. For instance, three sera (1st P#24, 2nd P#61 and 2nd P#217) reacted with alleles carrying 79GT which has a 97.9% frequency and they came from women who typed as A*24:02, A*24:02 (two times) or A24:02, A32:01; these alleles carry the Bw4-related epitope defined by the AbVer 80I.

Both antibody producers (1st P#49 and 2nd P#364) with antibodies reacting with alleles carrying 138MI (90.4%) were homozygous for A*02:01. Serum 1st P#228 which had antibodies reacting with alleles carrying 166EW+s65R+s158A (94.5%) came from a woman who typed as A*01:01, A*24:02; these alleles represent homozygosity for the AbVer 166DG epitope. Similarly, 1st P#228 and 2nd P#124 which reacted with 131S (97.1%) came from women who were homozygous for 131R and the reactivity with 113H (95.5%) of 2nd P#93 and 2nd P#348 came from women who were homozygous for 113Y.

Five sera reacted with 163EW-carrying alleles with three reactivity patterns that suggested a requirement of a distinct nearby amino acid configuration for this epitope namely (1) the AbVer 163EW+s65R+s158A epitope, (2) 163EW+s66I whereby 66N is permissible and (3) 163EW+s170R. There were two structurally similar epitopes defined by 170RH in combination with s103V and s62RN, respectively. These findings illustrate how amino acid variations within the context of a structural epitope around an eplet can affect epitope expression.

Discussion

The determination of HLA mismatch acceptability at the epitope level offers a more accurate approach to identify suitable donors for sensitized patients (Duquesnoy *et al.*, 2015). This can be best performed with epitopes that have been verified experimentally with informative antibodies. The website-based International Registry of HLA Epitopes (http://www.epregistry.com.br) describes the repertoires of antibody-verified epitopes on alleles encoded by the various HLA loci (Duquesnoy *et al.*, 2013). HLAMatchmaker offers structural descriptions of such epitopes; they correlate with eplets or eplets paired with nearby amino acid configurations. Although 81 antibody-verified HLA-ABC have been recorded in the HLA Epitope Registry, this list is incomplete and more studies need to be conducted with informative antibodies recognizing new epitopes.

Pregnancy-associated HLA sensitization 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. One can expect that most sera have antibodies that react with child-specific antibody-verified epitopes. The current studies focused on postpregnancy sera also reacting with alleles without child-specific antibody-verified epitopes. We hypothesized that such alleles would carry new epitopes that can be identified with HLAMatchmaker and determinations of critical amino acid residues within the context of structural epitopes defined by eplets. Our analysis has yielded sixteen putative new AbVer epitopes seven of which correspond to single eplets and nine are defined by combinations of eplets with other nearby residue configurations which appeared always be present in one or more alleles of the antibody producer. Such configurations are referred to as self.

Table 3. Newly antibody-defined HLA class I epitopes and their presence on HLA alleles in the current SAB panel

Epitope	Pregnancy Serum	Ep Freq	Alleles in SAB Panel Used for Antibody Analysis
$6K_2$	1stP#186	5.6%	C*01:02
66NV	2ndP#102, 2ndP#364	71.4%	A*03:01 A*11:01 A*11:02 A*25:01 A*26:01 A*29:01 A*29:02 A*30:01 A*30:02 A*31:01 A*32:01 A*33:01 A*33:03 A*34:02 A*43:01 A*66:01 A*66:02 A*68:01 A*68:02 A*69:01 A*74:01 A*80:01
79GT	1stP#24, 2ndP#61, 2ndP#217	97.9%	A*01:01 A*02:01 A*02:03 A*02:06 A*03:01 A*11:01 A*11:02 A*26:01 A*29:01 A*29:02 A*30:01 A*30:02 A*31:01 A*33:01 A*33:03 A*34:01 A*34:02 A*36:01 A*43:01 A*66:01 A*66:02 A*68:01 A*68:02 A*69:01 A*74:01 A*80:01
131S	1stP#228, 2ndP#124	91.7%	B*13:01 B*13:02 B*14:01 B*14:02 B*15:01 B*15:02 B*15:03 B*15:10 B*15:11 B*15:12 B*15:13 B*15:16 B*18:01 B*27:05 B*27:08 B*35:01 B*37:01 B*38:01 B*39:01 B*44:02 B*44:03 B*45:01 B*46:01 B*47:01 B*49:01 B*50:01 B*51:01 B*51:02 B*52:01 B*53:01 B*54:01 B*55:01 B*56:01 B*57:01 B*57:03 B*58:01 B*59:01 B*67:01 B*78:01 B*82:01
113H	2ndP#93, 2ndP#348	95.5%	B*07:02 B*08:01 B*13:01 B*13:02 B*15:01 B*15:03 B*15:10 B*15:11 B*15:12 B*15:16 B*18:01 B*35:01 B*38:01 B*39:01 B*40:01 B*40:02 B*40:06 B*41:01 B*42:01 B*46:01 B*48:01 B*51:01 B*51:02 B*52:01 B*53:01 B*54:01 B*55:01 B*56:01 B*57:01 B*57:03 B*58:01 B*59:01 B*67:01 B*78:01 B*81:01 B*82:01 C*15:02
138MI	1stP#49, 2ndP#364	90.4%	A*01:01 A*03:01 A*11:01 A*11:02 A*23:01 A*24:02 A*24:03 A*25:01 A*26:01 A*29:01 A*29:02 A*30:01 A*30:02 A*31:01 A*32:01 A*33:01 A*33:03 A*34:01 A*34:02 A*36:01 A*43:01 A*66:01 A*66:02 A*74:01 A*80:01
207S	1stP#228, 2ndP#61	77.6%	A*02:01 A*02:03 A*02:06 A*25:01 A*26:01 A*29:01 A*29:02 A*31:01 A*32:01 A*33:01 A*33:03 A*34:01 A*34:02 A*43:01 A*66:01 A*66:02 A*68:01 A*68:02 A*69:01 A*74:01 A*80:01
62LQ+s163T	2ndP#95	7.0%	A*29:01 A*29:02
76VRN+s152E/T/V	1stP#254	52.3%	B*46:01 B*73:01 C*01:02 C*03:02 C*03:03 C*03:04 C*08:01 C*12:03 C*14:02
80K+s14R	2ndP#430	44.4%	C*02:02 C*05:01 C*06:02 C*15:02 C*17:01 C*18:02
163EW+s66I/N	1stP#233, 2ndP#217	47.5%	A*66:02 B*07:02 B*13:01 B*13:02 B*27:05 B*27:08 B*40:01 B*40:02 B*40:06 B*47:01 B*48:01 B*73:01 B*81:01
163EW+s170R	2ndP#164, 2ndP#364	52.1%	A*66:02 B*07:02 B*13:01 B*13:02 B*27:05 B*27:08 B*40:01 B*40:02 B*40:06 B*47:01 B*48:01 B*73:01 B*81:01 C*02:02
163L+167S/G	1stP#24	27.3%	B*15:12 B*44:02 B*44:03 B*45:01 B*82:01
166EW+s65R+s158A	1stP#228	94.5%	A*02:01 A*02:03 A*02:06 A*03:01 A*11:01 A*11:02 A*24:03 A*25:01 A*26:01 A*29:01 A*29:02 A*30:01 A*30:02 A*31:01 A*32:01 A*33:01 A*33:03 A*34:01 A*34:02A*36:01A*66:01 A*66:02 A*68:01 A*68:02 A*69:01 A*74:01 B*15:16 B*57:01 B*57:03 B*58:01
170RH+s103V	2ndP#430	27.9%	A*33:01 B*14:01 B*14:02 B*18:01 B*52:01
170RH+s62RN	2ndP#61	26.5%	A*33:01 B*14:01 B*14:02 B*18:01 B*73:01

These studies required the identification of 'informative' reactive alleles that distinctly carried a putative new epitope, whereas all other nonreactive alleles lacked such epitope. Moreover, the new epitope must have a singular structural description based on polymorphic amino acid residues shared by all specifically reacting alleles. For some epitopes, we identified certain alleles that had a residue difference but they were equally antibody-reactive. Such permissible residue substitutions reflect a Landsteiner type of serological cross-reactivity as discussed elsewhere (Duquesnoy *et al.*, 2016). For other epitope-carrying alleles, a residue difference was associated with less reactivity (a lower MFI value). Such alleles could still be considered as reactive, but obviously, these different residues have an inhibitory effect.

Epitopes defined by eplet pairs have certain residues that are critical for reactivity with antibody. Other residues in the same sequence position render a nonreactivity with antibody as assessed by a threshold MFI value.

A potential limitation of this study is the choice of the MFI = 2000 cut-off point with the immunizing allele as a way to select well-reacting sera to identify informative alleles expressing new epitopes. Although most of them have relatively high MFI values, the SAB panel might have other less-reactive alleles which introduce a certain level uncertainty in the analysis. Another limitation is that no serum absorption-elution studies with selected alleles were carried out and no monoclonal antibodies were generated. It relied solely on the interpretation of reactivity patterns with an

HLA-typed allele panel, a practice routinely used in the clinical setting for the epitope analysis of serum antibodies to determine mismatch acceptability.

We conclude that analyses such as these provide informative data on antibody-related epitopes. This information will be added to the International Registry of HLA Epitopes website for use by HLA professionals in their efforts to determine HLA mismatch acceptability.

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 conflict of interest to disclose.

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