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Brief communication

Brief report: Why did two patients who type for HLA-B13 have antibodies that react with all Bw4 antigens except HLA-B13?

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ABSTRACT

Two transplant candidates sensitized during pregnancy by a B*44:02 mismatch showed antibodies that reacted with an epitope defined by the 145R + 82LR eplet pair shared by all Bw4 antigens in single allele Luminex panels except B13. Both eplets are on one or more alleles of the antibody producer and according to HLAMatchmaker, they are considered intralocus and interlocus matches which should not induce antibodies. The recently developed nonself-self paradigm for HLA epitope immunogenicity has offered a ready explanation why the pair of self-145R and self-82LR eplets on B*44:02 induced specific antibodies. This finding is consistent with the concept that alloantibody responses originate from B-cells with self-HLA immunoglobulin receptors.

1. Introduction

Serum screening for HLA antibodies is important for the determination of mismatch acceptability for sensitized patients. Interpretations of serum reactivity patterns with HLA panels should consider the fact that HLA antibodies are specific for epitopes rather than antigens or alleles. HLAMatchmaker is a computer algorithm designed to determine HLA compatibility at the epitope level and to analyze serum reactivity for HLA epitope-specific antibodies [1,2]. It considers small patches of polymorphic amino residues referred to as eplets as essential components of HLA epitopes. Notations of eplets consist of amino acid sequence numbers and polymorphic residue descriptions with standard single letter codes. Certain sera have unexpected reactivity patterns with HLA panels that can be explained with epitope-specific antibodies. For instance, a recent report describes a transplant recipient who typed as B*44:03 had antibodies that reacted with B*44:02 [3]. This patient was sensitized by the donor's C*07:04 which carries a mismatched epitope defined by the 156DA eplet which is shared with B*44:02 and a group of HLA-B alleles including B*08:01, B*37:01, B*41:01, B*42:01, B*45:01, and B*82:01, all of them reacted with patient's serum. Another study addressed the question why patients sensitized by a DR2 mismatch may have antibodies that react also with DR1 [4]. Although these antigens might share a distinct epitope recognized by these antibodies, HLAMatchmaker cannot predict such structurally defined epitope. Nevertheless, the reactivity with DR1 can be readily explained with antibodies induced by DR51 which is in strong linkage disequilibrium with DR2. They are specific for the 96EV eplet shared between DR51 and DR1. Conversely, sensitization by a DR1 mismatch can lead to antibodies that react also with DR51 but not with DR2. These findings show that 96EV represents a highly immunogenic epitope that can induce cross-sensitization between antigens encoded by different DRB loci. It has become apparent that sensitization induced by an epitope on an HLA mismatch may cause other HLA antigens to become unacceptable mismatches because they share that epitope although the patient may have never been exposed to such antigens [5].

This report describes two cases with HLA class I antibodies with specificities that appeared to be related to Bw4, a well-known public antigenic determinant shared by a group of HLA-B antigens plus HLA-A23, A24, A25 and A32. Normally, only patients who type for Bw6 and lack these HLA-A antigens can develop Bw4-specific antibody responses but this report describes two kidney transplant candidates with unexpected antibodies reacting with all Bw4-carrying antigens except B13. Both patients type for B13 and had been sensitized by a Bw4-carrying B44 mismatch during pregnancy. We have used HLAMatchmaker to explain this unexpected antibody reactivity pattern. Bw4 is equivalent to the 82LR eplet represented by a leucine and an arginine residue in sequence positions 82 and 83 [1]. Although 82LR is on the sensitizing B44 it cannot be considered a non-self eplet because both patients type for B13 which has also 82LR. Given the fact

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that certain HLA antibodies are specific for eplet pairs, we reanalyzed these sera and results show antibody specificity for a class I epitope defined by an eplet pair: 145R + 82LR. Interestingly, 145R can also be considered self because this eplet is present in the HLA types of the antibody producers but as described below, the recently developed nonself-self paradigm for HLA epitope immunogenicity [6] offers a ready explanation.

2. Materials and methods

Both patients were first kidney transplant candidates. Patient 1 was a 52-year old female with focal glomerulosclerosis. She typed as HLA-A*30:01, A*66:01; B*13:02, B*14:02; C*06:02, C*08:02 and had three pregnancies from a husband who typed as HLA-A*03:01, A*68:01; B*18:01, B*44:02. No blood transfusions had been given. Patient 2 was a 66 year-old female with cystic nephropathy. She typed as HLA-A*02:01, A*11:01; B*07:02, B*13:02; C*06:02, C*07:02 and had two pregnancies from a husband who typed as HLA-A*11:01, A*24:02: B*18:01, B*44:02. She received one red blood transfusion in 1993.

Serum samples were tested with Luminex microbead assays using single HLA-ABC allele kits from a commercial vendor (One Lambda Inc., Canoga Park, CA) according to manufacturers' instructions. Briefly, small volumes of patient serum were incubated with a mixture of Luminex microspheres, each coated with a single HLA class I allele, in 96-well plates. After washing to remove unbound antibody, the mixtures were incubated with phycoerythrin-conjugated anti-human IgG. After incubation, the wells were diluted and analyzed on a Luminex 100 instrument (Luminex, Austin, TX). Median fluorescence intensity (MFI) values were determined using the manufacturer's software. Reactivity was determined by comparing the MFI values of the beads possessing individual mismatched antigens to those of positive and negative controls, as well as to the average MFI of beads possessing the patients' self HLA antigens.

We analyzed antibody reactivity patterns with the recently developed eplet pair-based HLAMatchmaker antibody analysis program which has worksheets to enter the HLA types of the antibody producer, the immunizer and the allele panel as well as the MFI values that can be readily copied from the manufacturer's Luminex computer files. A new feature is the determination of immunizer-specific and third-party eplets and eplet pairs shared by antibody-reactive alleles. This program is posted on the website http://www.HLAMatchmaker.net.

3. Results and discussion

Two serum samples obtained from each patient on different dates (Patient 1: September 2009 and August 2010; Patient 2: December 2010 and January 2011) and tested in Torino and Pittsburgh showed similar reactivity patterns with single allele Luminex panels. Table 1 shows the average median MFI values. B*44:02 was an immunizing allele for both patients. All reactive alleles in the Luminex panel shared two epitopes with B*44:02, namely the eplet pair represented by 145R + 82LR on a large group of alleles and 166ES a commonly reactive eplet on B*44:02, B*44:03, B*45:01 and B*82:01. The sera from patient 1 reacted weakly with 166ES. This patient had also A*68:01-induced antibodies against 138MT, an immunogenic eplet shared between A2, A68 and A69. Patient 2 did not have 138MT-reactive antibodies. There were a few extra reactions namely with A*01:01 (patient 1) and B*15:12 (both patients) which cannot be explained. The remaining alleles including self-alleles had very low MFI values and none of them had the epitopes described above.

The epitope defined by 145R + 82LR presented the major challenge of this study, because both components of this pair represent self-eplets for these patients. The B*13:02 allele of both of them has 82LR whereas A*30:01, A*66:01 and B*14:02 of patient 1 and A*11:01, B*07:02, C*06:02 and C*07:02 of patient 2 have 145R. These eplets themselves are considered intralocus and interlocus matches and according to HLAMatchmaker they should not induce humoral immunity [1,7]. However, it seems likely that the combination of 145R and 82LR on the immunizing B*44:02 must be considered a mismatched epitope that had elicited an antibody response. Recent studies by El-Awar et al. have verified the existence of a very similar epitope (#249) defined by a murine monoclonal antibody that react with all Bw4-associated antigens except B13 [8]. Moreover, an abstract presentation report in 2007 described three cases with antibodies against an epitope defined by 82L, 83R and 145R [9]. For our two patients the nonself-self paradigm for HLA epitope immunogenicity [6] offers a ready explanation why the pair of self-145R and self-82LR eplets on B*44:02 induced specific antibodies.

This paradigm is based on the hypothesis that HLA antibodies originate from B-lymphocytes with low-avidity immunoglobulin receptors for self HLA epitopes [6]. Their interactions with self HLA proteins will not lead to B-cell activation or antibody production. In contrast, exposure to HLA mismatches induces often strong alloantibody responses. The activation of self-HLA specific B-cell by a nonself eplet would require that the remainder of the structural epitope of the immunizing antigen has considerable structural similarity with one of the antibody producer's alleles. Previous molecular modeling studies with six epitopes defined by human monoclonal antibodies have demonstrated that in each case, one allele of the antibody producer has no or few differences with the immunizing allele in antibody-accessible positions defined by a 15 Å radius of the mismatched eplet. Considering the 700–900 $Å^2$ surface of a structural

Table 1
Serum reactivity patterns with single allele Luminex panels.

	Patient 1	Patient 2	Epitopes
	MFI	MFI	
Positive control	6314	8852	
Negative control	11	33	
Self Alleles	45 ± 23	96 ± 45	
B*44:02 IMM	2246	2636	145R+82LR/166ES
A*23:01	2635	3400	145R+82LR
A*24:02	2303	2278	145R+82LR
A*24:03	2266	2453	145R+82LR
A*25:01	1270	1625	145R+82LR
A*32:01	2764	3365	145R+82LR
B*15:13	1582	1781	145R+82LR
B*15:16	2078	2130	145R+82LR
B*27:05	2732	3217	145R+82LR
B*37:01	2259	4593	145R+82LR
B*38:01	2280	2795	145R+82LR
B*44:03	2326	3034	145R+82LR/166ES
B*47:01	1750	1641	145R+82LR
B*49:01	2634	3688	145R+82LR
B*51:01	1888	2252	145R+82LR
B*51:02	2467	3127	145R+82LR
B*52:01	1958	2705	145R+82LR
B*53:01	2521	2938	145R+82LR
B*57:01	1904	2626	145R+82LR
B*57:03	1964	2655	145R+82LR
B*58:01	1551	2035	145R+82LR
B*59:01	2255	2787	145R+82LR
B*45:01	442	2263	166ES
B*82:01	125	3820	166ES
A*68:01 IMM	1110	71	138MT
A*02:01	671	128	138MT
A*02:03	798	69	138MT
A*02:06	843	97	138MT
A*68:02	1064	212	138MT
A*69:01	777	79	138MT
A*01:01	2537	125	Third party?
B*15:12	413	520	?
Other alleles	43 ± 18	106 ± 46	

epitope one can estimate that surface residues within 15 Å of a mismatched eplet could make contact with a given antibody. These residues can be identified with the "select by distance" command of the Cn3D structure software program [10] using informative HLA models downloaded from Entrez Molecular Modeling Database on the National Center for Biotechnology Information website: http://www.ncbi.nlm.nih.gov/Structure.

Table 2 shows the results of a comparative analysis of surface residues within a 15 Å radius of the 145R eplet on the immunizing B*44:02 and the alleles of the antibody producers. Amino acid residue differences in antibody-accessible polymorphic positions are marked with an exclamation symbol (!). The immunizing B*44:02 has 145R which is a mismatch for B*13:02 which has 145L. It should be noted that only B*13:02 has the 82LR part of the 145R + 82LR epitope on B*44:02; all other alleles of the antibody producer have 82RG. Furthermore, B*13:02 and B*44:02 have no amino acid differences within 15 Å of 145R. In contrast, the other alleles of these antibody producers have multiple differences ranging from 4 to 10. This means that the structural epitopes of the immunizing B*44:02 and the antibody producer's B*13:02 are identical except for the arginine and leucine difference in sequence position 145. In this case 145R can be considered the driving force of the antibody response to the 145R + 82LR epitope.

The locations of 145R and surrounding residues on a crystalline model of the molecular structure of B*44:02 are depicted in Fig. 1. The yellow area represents the molecular surface within a 15 Å radius of 145R; the numbers indicate sequence locations and standard letter codes indicate polymorphic residues shown in Table 2 all of them are identical for B*13:02. The antibody defined epitope consists of 145R which is nonself for B*13:02 and paired with self 82LR; these eplets are about 13 Å apart a sufficient distance for contact by two separate Complementarity Determining Regions (CDRs) of antibody.

These findings suggest that 145R + 82LR-specific antibodies in these two patients originated from B-cells with low avidity immunoglobulin receptors specific for the structurally defined 145 L epitope on self B*13:02. Such receptors might interact though their CDRs with different portions of the B*13:02 molecular surface but the binding strength is so weak that B-cell activation and antibody production cannot occur. In contrast, a substitution of 145 L by 145R within this structural epitope would trigger the activation of these B-cells and antibody responses in these patients. The immunizing B*44:02 has this 145R difference with B*13:02 whereas all other surface residues in the structural epitope are identical. In particular, the self 82LR eplet on B*13:02 and shared with B*44:02 is critical for antibody binding and this explains how this epitope can be defined by the 145R + 82LR pair.

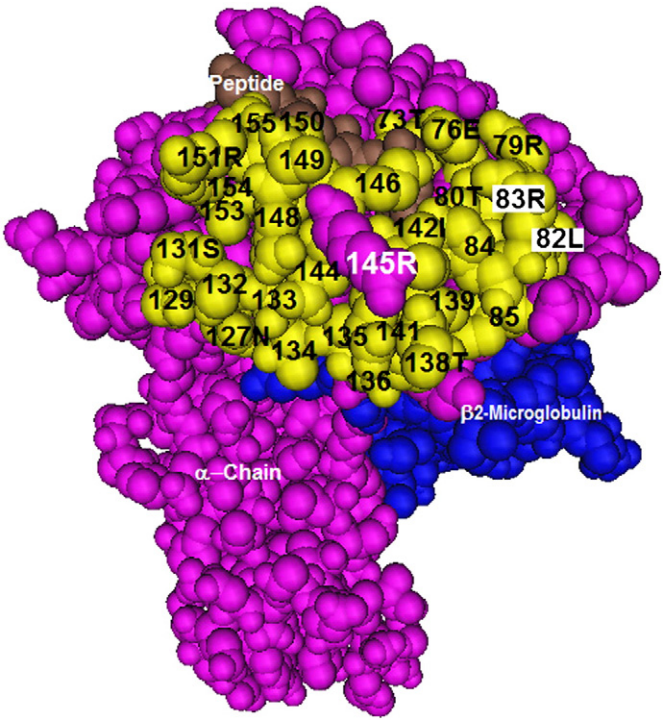

Fig. 1. Molecular model of B*44:02 showing surface-exposed residues surrounding 145R (in white font) within a 15 Å radius (in yellow). Single numbers refer to sequence locations of monomorphic residues and numbers with letters describe polymorphic residues shared between B*44:02 and B*13:02. Self residues 82 L and 83R are essential components of the epitope.

In summary, this study is an example how HLAMatchmaker in combination with the nonself-self algorithm of HLA epitope immunogenicity can explain unexpected HLA antibody reactivity patterns in sensitized patients. This finding is consistent with the concept that alloantibody responses originate from B-cells with self-HLA immunoglobulin receptors.

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Table 2
Polymorphic surface residue differences in the 15 Å^2 region surrounding 145R on the immunizing B*44:02 and the alleles of the antibody producers.

Residue positions	145	Number of differences	73	76	79	80	82	83	127	131	138	142	144	149	151	152
B*44:02 Immunizer	[R]		T	E	R	T	L	R	N	S	T	I	Q	A	R	V
Patient 1																
A*30:01	R	7	–	V	G	–	R	G	–	R	M	–	–	–	–	W
A*66:01	R	9	–	V	D	–	R	G	–	R	M	–	–	T	H	E
B*13:02	[L]	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B*14:02	R	4	–	–	–	N	R	G	–	–	–	–	–	–	–	E
C*06:02	R	7	A	V	–	K	R	G	–	R	–	–	–	–	–	E
C*08:02	R	6	–	V	–	N	R	G	–	R	–	–	–	–	–	E
Patient 2																
A*02:01	H	10	–	V	G	–	R	G	K	R	M	T	K	–	H	–
A*11:01	R	9	–	V	G	–	R	G	–	R	M	–	K	–	H	A
B*07:02	R	5	–	–	–	N	R	G	–	R	–	–	–	–	–	E
B*13:02	[L]	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–
C*06:02	R	7	A	V	–	K	R	G	–	R	–	–	–	–	–	E
C*07:02	R	7	A	V	–	N	R	G	–	R	–	–	–	–	–	A

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