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Highlights

*Transplant Immunology xxx (2015) xxx – xxx***HLA-A2 reactive antibodies in a patient who types as HLA-A2: The importance of high resolution typing and epitope-based antibody analysis**A.B. Hahn^{a,*}, V. Bravo-Egana^a, J.L. Jackstadt^a, D.J. Conti^b, R.J. Duquesnoy^c^a Transplantation Immunology Laboratory, Department of Surgery, Albany Medical College, Albany, NY, *United States*^b Section of Transplantation, Department of Surgery, Albany Medical Center, Albany, NY, *United States*^c Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, *United States*

- A patient's apparent antibody to self antigen was allele specific.
- The responsible epitope could not be solved by HLA Matchmaker alone.
- The nonself-self paradigm augmented epitope-based antibody specificity analysis.
- A new antibody-verified epitope 43Q + 62GER was identified.
- Allele level typing aids identification of suitable donors for sensitized patients.



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Brief communication

Q1 HLA-A2 reactive antibodies in a patient who types as HLA-A2: The importance of high resolution typing and epitope-based antibody analysis

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ABSTRACT

This report describes a case of a highly sensitized patient who had serum antibodies reacting with HLA-A2 but whose phenotype included HLA-A2. The determination of HLA mismatch acceptability at the antigen level was problematic, but high-resolution HLA typing information and epitope-based antibody specificity analysis based on the nonself-self paradigm of HLA epitope immunogenicity have provided a solution. This case supports the concept that HLA typing at the allele level offers a better approach to identifying suitable donors for sensitized patients.

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1. Introduction

Sensitized transplant candidates have serum antibodies that react with HLA antigens which are then traditionally considered as unacceptable mismatches. HLA antibodies are now recognized as being specific for epitopes which can be defined structurally with amino acid differences between HLA alleles. The determination of mismatch acceptability should therefore be based on epitopes rather than antigens. A recent "Personal Viewpoint" paper addresses the concept that HLA typing at the four-digit or allele level offers a more accurate approach to identify suitable donors for sensitized patients (1).

Antibodies have six complementarity determining region loops that interact with structural epitopes consisting of 15–25 amino acid residues distributed on a molecular surface of 700–900 Å² (2). Each structural epitope has a certain residue configuration referred to as a functional epitope which dominates the binding with antibody.

The nonself-self paradigm of HLA epitope immunogenicity is based on the hypothesis that B-lymphocytes carry low-affinity immunoglobulin receptors for self-HLA epitopes (3). Their interactions with self-HLA will not lead to B-cell activation or antibody production. In contrast,

exposure to HLA mismatches can induce a strong alloantibody response which is the result of a productive interaction of the immunoglobulin receptor with a non-self residue configuration whereby the remainder of the structural epitope on the immunizing antigen must be identical or very similar to the corresponding self HLA epitope of the antibody producer. Recent observations have demonstrated the usefulness of nonself-self paradigm of HLA epitope immunogenicity in the antibody verification of HLA epitopes (4–7).

2. Case description

A 41 year-old African-American female patient with no pregnancies and 5 transfusions types as HLA-A2,A3; B7,B45; Cw7,Cw16; DR11,DR15. She had received a kidney transplant in 1998 from a zero-HLA-A,B,DR antigen mismatch deceased donor: HLA-A2,–; B7,B45; Cw6,Cw7; DR11,DR15 and in 2000 she received a zero-HLA-A,B,C,DR antigen mismatch pancreas transplant: HLA-A3,–; B7,–; Cw7,–; DR15,–. Both transplants failed eventually and in November 2014 the patient was evaluated for a possible second kidney transplant.

Serum screening with single allele Luminex beads (LABScreen Single Antigen, One Lambda, ThermoFisher) showed positive reactions with all three HLA-A2 alleles, HLA-A80 and the following HLA-C antigens: Cw2, Cw4, Cw5, Cw6, Cw12, Cw15, Cw17, Cw18. Reactivity to multiple DP alleles was also found. With a calculated Panel- Reactive Antibody (cPRA)

Abbreviations: cPRA, calculated panel reactive antibody; MFI, mean fluorescence intensity.

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of 84%, this patient can be considered highly sensitized. Two subsequent tests on the same serum confirmed these reactivity patterns.

3. HLA-A epitope specificity analysis

High-resolution typing (Micro SSP Allele Specific HLA Class I DNA Kit, One Lambda, ThermoFisher, Inc.) showed that the patient's HLA-A2 corresponds to A*02:02 and we postulated that the reactive Luminex alleles A*02:01, 02:03 and 02:06 must have a distinct epitope which is absent on A*02:02. The current version of HLA-Matchmaker was not able to identify this epitope because it assumes that an individual cannot form antibodies against self targets. Thus, shared amino acid sequences are treated as self and not considered in the algorithm.

A comparative analysis of amino acid sequences showed that the patient's A*02:02 has a residue difference with the antibody-reactive A*02:01, A*02:03, A*02:06 in the antibody-accessible position 43 on the molecular surface, namely 43R versus 43Q. All other HLA-A alleles in the Luminex panel have 43Q and, with the exception of A*80:01, they do not react with patient's antibodies. They include the patient's own A*03:01, which indicates that 43Q is a self-residue. These findings raised the question of how this antibody specificity can be defined by 43Q.

No high-resolution typing data were available for the kidney donor in 1998, but we assume in our analysis that the high-frequency A*02:01 would likely be the immunizing allele (a consideration of A*02:03 and A*02:06 would not make any difference). We propose that the HLA-A2-reactive antibodies must have originated from B-cells with immunoglobulin receptors specific for a self-epitope on the patient's A*02:02, which has 43R, and other nearby self residues. These B-cells were activated by a nonself 43Q-defined epitope on the immunizing A*02:01 in context with a nearby self residue configuration within the structural epitope. All antibody-reactive 43Q-carrying alleles must have that configuration, whereas the nonreactive 43Q-carrying alleles, including the patient's A*03:01, have a different configuration.

Table 1 shows the mean fluorescence intensity (MFI) values for the HLA-A alleles in the One Lambda Luminex panel as well as amino acid descriptions in sequence positions 43, 62, 63 and 65 which are important in defining the epitope detected by the patient's antibodies.

All HLA-A alleles in the single antigen panel have 43Q, and the antibody-reactive A*02:01, A*02:03 and A*02:06 have a second unique configuration 62G + 63E + 65R which is shared with the antibody producer's A*02:02 but absent on all remaining non-reactive HLA-A alleles. All HLA-B alleles are non-reactive; they carry 43P. These findings suggest that the patient's antibodies recognize an epitope which can be defined by the 43Q + 62G + 63E + 65R combination.

The A*80:01 bead had MFI values ranging from 1282 to 2324 when tested in triplicate. There are two possible explanations for this weak reactivity. First, A*80:01 has been reported to have non-HLA related reactivity with so-called natural antibodies (8,9) and our experience with other patient sera has shown that this allele often has this unexpected reactivity. Second, A*80:01 has a 62E + 63E + 65R configuration which is unique in this Luminex panel and it is possible that the difference of 62E versus 62G reflected a permissible residue substitution which led to a lower but still positive MFI value reflecting this epitope specificity.

Subsequent testing with an expanded Luminex panel (Supplement Group 1, LAB Screen Single Antigen, ThermoFisher, One Lambda) showed, as predicted, positive reactions for the 43Q + 62GER-carrying A*02:07 (MFI = 1093) and A*02:10 (MFI = 3082) whereas A*02:05, which has 43R, was negative (MFI = 395). All other HLA alleles were nonreactive (MFI = 101 ± 180, N = 31) except the 80K-carrying C*18:01 (MFI = 11,596).

Altogether, these findings suggest that this patient's antibodies reacted with an epitope determined by residues 43Q + 62G + 63E + 65R. According to the eplet notations in HLA-Matchmaker, this epitope is called

Table 1
Epitope specificity analysis of antibody reactivity with HLA-A and HLA-B alleles.

Patient Phenotype	Self	MFI	Epitope 43Q+62G+63E+65R			
			Nonself for A*02:02	Self for A*02:02		
			not done	43R	62G	63E
A*02:02	Self					
A*03:01	Self	187	43Q	62Q	63E	65R
B*07:02	Self	211	43P	62R	63N	65Q
B*45:01	Self	210	43P	62R	63E	65Q
C*07:02	Self	308	43P	62R	63E	65Q
C*16:01	Self	246	43P	62R	63E	65Q
A*02:01	Immunizer	6631	43Q	62G	63E	65R
A*02:03		5561	43Q	62G	63E	65R
A*02:06		4542	43Q	62G	63E	65R
A*80:01		2324	43Q	62E	63E	65R
A*01:01		426	43Q	62Q	63E	65R
A*11:01		442	43Q	62Q	63E	65R
A*11:02		532	43Q	62Q	63E	65R
A*23:01		284	43Q	62E	63E	65G
A*24:02		376	43Q	62E	63E	65G
A*24:03		759	43Q	62E	63E	65G
A*25:01		549	43Q	62R	63N	65R
A*26:01		470	43Q	62R	63N	65R
A*29:01		471	43Q	62L	63Q	65R
A*29:02		378	43Q	62L	63Q	65R
A*30:01		187	43Q	62Q	63E	65R
A*30:02		221	43Q	62Q	63E	65R
A*31:01		218	43Q	62Q	63E	65R
A*32:01		266	43Q	62Q	63E	65R
A*33:01		196	43Q	62R	63N	65R
A*33:03		183	43Q	62R	63N	65R
A*34:01		620	43Q	62R	63N	65R
A*34:02		533	43Q	62R	63N	65R
A*36:01		337	43Q	62Q	63E	65R
A*43:01		506	43Q	62L	63Q	65R
A*66:01		646	43Q	62R	63N	65R
A*66:02		329	43Q	62R	63N	65R
A*68:01		210	43Q	62R	63N	65R
A*68:02		323	43Q	62R	63N	65R
A*69:01		287	43Q	62R	63N	65R
A*74:01		241	43Q	62Q	63E	65R
Negative HLA-A alleles		384 ± 159	43Q			
All HLA-B alleles (N = 50)		253 ± 87	43P			

43Q + 62GER. These configurations are about 8 Å² apart and would be contacted by different CDRs of antibody.

By definition, an antibody-reactive allele in the Luminex panel is an unacceptable mismatch. Most non-Luminex HLA-A2 alleles can also be considered unacceptable mismatches because they carry the 43Q + 62GER epitope recognized by the patient's antibodies; A*02:70 would be an exception because this allele has 43Q + 62GEG. Conversely, A*02:05, A*02:08, A*02:14, A*02:47, A*02:63, A*02:115, A*02:154 and A*02:155 could be considered acceptable mismatches because, like the antibody producer's A*02:02, they have 43R. The vast majority of non-A2 HLA-A alleles have 43Q, but they are acceptable mismatches because they lack the 62GER configuration necessary for the epitope. However, there are some exceptions, including A*03:23, A*24:08, A*24:42, A*24:89, A*26:07, A*33:08, A*33:09, A*68:30 and A*74:04 because they have 43Q + 62GER epitope.

It should be pointed out that the determination of mismatch acceptability of non-Luminex alleles is based only on theoretical considerations about sharing of eplets and eplet pairs. Some alleles may have additional amino acid differences which might affect reactivity with antibody. A physical crossmatch should be performed to verify lack of antibody reactivity and donor/recipient compatibility.

4. HLA-C epitope specificity analysis

HLA-Matchmaker confirmed that most of the HLA-C reactivity (Table 2) was due to antibodies specific for the well-documented 80K-defined epitope presented by C*06:02 of the previous kidney transplant.

We noted that the 80K-carrying C*15:02 had a much lower, but still significant, reactivity (MFI = 4364) than the other 80K-carrying alleles. The presence of 66N in C*15:02 rather than the 66K in all other 80K-

Table 2

Epitope specificity analysis of antibody reactivity with HLA-C alleles.

Patient Phenotype	MFI	First epitope:80K		Second epitope:73A+147W	
			Non-self for C*16:01	Self for C*16:01	
A*02:02 Self	nd	80T	73T	147W	
A*03:01 Self	187	80T	73T	147W	
B*07:02 Self	211	80N	73T	147W	
B*45:01 Self	210	80N	73T	147W	
C*07:02 Self	308	80N	73A	147L	
C*16:01 Self	246	80N	73T	147W	
C*06:02 Immunizer	14,000	80K	73A	147W	
C*04:01	10,831	80K	73A	147W	
C*18:02	13,688	80K	73A	147W	
C*17:01	14,413	80K	73A	147L	
C*05:01	16,325	80K	73T	147W	
C*02:02	15,727	80K	73T	147W	
C*15:02	4364	80K	73T	147W	
C*12:03	4185	80N	73A	147W	
C*01:02	226	80N	73T	147W	
C*03:02	235	80N	73T	147W	
C*03:03	256	80N	73T	147W	
C*03:04	256	80N	73T	147W	
C*08:01	242	80N	73T	147W	
C*14:02	261	80N	73T	147W	
C*16:01	246	80N	73T	147W	

carrying alleles (not shown) might be responsible for lowering the antibody binding strength and MFI.

In addition to the reactive 80K-carrying HLA-C alleles on the Luminex panel, non-Luminex 80K-carrying HLA-C alleles such as C*01:14, C*03:07, C*03:10, C*07:07, C*07:09, C*08:10, C*12:04 and C*12:05 would be unacceptable mismatches. Conversely, the 80N-carrying C*02:27, C*04:11, C*05:20, C*06:11 and C*15:07 appear to be acceptable mismatches.

In addition to the 80K-defined epitope, a new epitope 73A + 147W was defined by applying the nonself-self algorithm of HLA epitope immunogenicity. We postulate that this antibody originated from B-cells with immunoglobulin receptors specific for a self epitope on C*16:01 which included 73T and 147W, and that such B-cells were activated by the nonself epitope on C*06:02 defined by 73A + 147W. The Luminex panel had three additional 73A + 147W-carrying alleles, but C*04:01 and C*18:02 also have the immunogenic 80K epitope and thus were not informative. However, the 80K-negative C*12:03 allele was informative for the demonstration of the 73A + 147W specific antibody reactivity. These residues are 7.4 Å apart and would be contacted by different CDRs of antibody.

The non-Luminex alleles C*01:17, C*02:12, C*03:15, C*07:03, C*08:05, C*14:04 and C*15:03 also have the 73A + 147W epitope and can be considered unacceptable mismatches, but the 73T-carrying C*04:10, C*06:05, C*12:05 would be acceptable.

Again, in the absence of beads carrying these alleles, a physical cross-match is important to assess antibody reactivity.

5. Discussion

This report illustrates the importance of high-resolution HLA typing information and the usefulness of epitope-based antibody specificity analysis to understand the degree of humoral HLA sensitization and the determination of acceptable mismatches for sensitized transplant candidates. With a cPRA of 84% this patient can be considered highly sensitized and HLA antigen-based acceptable mismatching is problematic because her serum has HLA-A2 reactive antibodies but her phenotype has HLA-A2. This high serum reactivity to class I antigens can be entirely explained with antibodies specific against three epitopes apparently presented by mismatched HLA of a previous transplant: 43Q + 62GER on HLA-A and 80K and 73A + 147W on HLA-C. HLAMatchmaker readily identified the 80K antibody specificity but it did not work for the other two epitopes. HLAMatchmaker considers

epitopes defined by one or few polymorphic residues as essential components of epitopes, and donor-recipient matching is determined through intralocus and interlocus comparisons of such eplets. Shared amino acid sequences are treated as self and not considered in the algorithm. Although this approach is effective for the epitope specificity analysis of most HLA antibodies, this report and two other publications (5,7) demonstrate that there are exceptions whereby eplets that are intralocus or interlocus matches can induce specific antibodies.

Such epitopes can be identified by using an antibody specificity analysis based on the nonself-self paradigm of HLA epitope immunogenicity, which considers the hypothesis that HLA antibodies originate from B-cells with low-affinity immunoglobulin receptors for self HLA epitopes on each allele of the antibody producer (2). Such B-cells can be activated by a mismatched HLA allele presenting an amino acid configuration (eplet) which is nonself for a given allele of the antibody producer whereas the remainder of the structural epitope must consist of self residues. Subsequent production of antibodies is accompanied by affinity maturation which increases antibody binding strength with certain self components of the epitope. Accordingly, such epitopes are defined by combinations of nonself and self residues. Because these combinations become intralocus and interlocus mismatches, they can be converted to eplet pairs and incorporated in HLAMatchmaker for antibody analysis. In this case, the 43Q + 62GER epitope can be recorded in the International HLA Epitope registry as being antibody-verified.

Because most HLA-A2-carrying organ donors would likely have the more common 43Q alleles of HLA-A2, and because only antigen equivalents can be entered into the UNet system as patient HLA antigens and unacceptable antigens, it was decided to enter HLA-A2 as an unacceptable antigen for this patient despite it being a self antigen.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the journal Transplant Immunology.

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