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Highlights

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 HLAMatchmaker 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

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-Egan 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 College, Albany, NY, United States
c Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, PA, United States

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 better approach to identifying 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,DR60 antigen mismatch deceased donor: HLA-A2,−; B7, B45; Cw6, Cw7; DR11, DR15 and in 2000 she received a zero-HLA-A,B, CDR 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-A 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)...
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 HLAMatchmaker 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 immuno-nizing 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*02:02 (MFI = 1093) and A*02:01 (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 diplet notations in HLAMatchmaker, this epitope is called 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 + 62EGE. 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*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

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

<table>
<thead>
<tr>
<th>Patient Phenotype</th>
<th>First epitope: 80K</th>
<th>Second epitope: 73A+147W</th>
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<tbody>
<tr>
<td>A*02:02 Self</td>
<td>nd</td>
<td>73T</td>
</tr>
<tr>
<td>A*03:01 Self</td>
<td>155</td>
<td>73T</td>
</tr>
<tr>
<td>C*07:02 Self</td>
<td>191</td>
<td>73T</td>
</tr>
<tr>
<td>B*05:01 Self</td>
<td>330</td>
<td>73T</td>
</tr>
<tr>
<td>C*01:02 Self</td>
<td>246</td>
<td>73T</td>
</tr>
<tr>
<td>C*06:02 Immunezer</td>
<td>14,000</td>
<td>73A</td>
</tr>
<tr>
<td>C*04:01</td>
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<td>73A</td>
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<tr>
<td>C*07:01</td>
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<tr>
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<td>C*12:03</td>
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<tr>
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<td>73A</td>
</tr>
<tr>
<td>C*16:01</td>
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<td>73T</td>
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First epitope: 80K, second epitope: 73A+147W.

Table 2

Discussion

This report illustrates the importance of high-resolution HLA typing and epitope-based antibody 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-A 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 + 62GCR 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 eplets 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 mismatches 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 + 62GCR 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 in the International HLA Epitope registry as being antibody-verified.

References

3. Duquesnoy RJ. HLA-A2 reactive antibodies in a patient who types as HLA-A2: The importance of high resolution typing and epitope-based antibody analysis, Transpl Immunol (2015), http://dx.doi.org/10.1016/j.trim.2015.04.001