Recognized and Unrecognized Sensitization
Assessment of pre-transplant immunologic memory

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No financial relationships related to this presentation

AND

The presentation *does not* include discussion of “off-label” or “investigational” use.
The presence of preformed cytotoxic antibodies against the donor appears to be a strong contraindication for transplantation.

"..the ethics of transplanting kidneys without the prior knowledge of the results of the lymphocyte crossmatch test... can reasonably be expected to be questioned."
The evolution and clinical impact of Human Leukocyte Antigen technology

Howard M. Gebel and Robert A. Bray

Current Opinion in Nephrology and Hypertension 2010, 19:598–602

- Cumbersome cell-based assays for XM, antibody ID and HLA typing.

- Highly sensitive and specific bead-based assays for antibody ID and Molecular-based HLA typing.

Figure 1 Evolution of human leukocyte antigen antibody testing.

Cytotoxicity
Solid Phase HLA antibody detection

HLA alloantibody

Anti-IgG-PE

Adapted from Gebel and Bray. Transplantation Reviews 20: 189-194, 2006
Antibody profile of three potential transplant candidates

No Class I antibodies

Class I

UNSENSITIZED??

No Class II antibodies

Class II
The Details
(The Devil is in them…)

1. Non-transfused male-Unsensitized
2. Multiparous female, 3 children
   Exposed to mismatched paternal ags
   but
   Unsensitized or Sensitized?
3. Previous allograft recipient
No Class I antibodies

No Class II antibodies
Horse hospitals
Alloreactive memory can arise not just from prior HLA sensitization, but also from pathogen exposure.
Donor-Reactive Memory T Cells are a Barrier to Success in Transplantation


Published by AAAS
How do we identify and quantify alloreactive memory T cells?

Clinical transplantation: presence of donor-specific memory T cells before transplantation correlates with the risk of post transplant acute rejection episodes and decreased graft function

Augustine et al., AJT 2005

Acute rejection episodes in the first 12 months post transplant

Kidney graft function at 12 months post transplant

IFNγ spots/300,000 PBMC:

<table>
<thead>
<tr>
<th></th>
<th>&gt; 25</th>
<th>&lt; 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>% patients</td>
<td>7/14</td>
<td>4/23</td>
</tr>
<tr>
<td>p</td>
<td>0.036</td>
<td></td>
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</table>

GFR, ml/min

<table>
<thead>
<tr>
<th></th>
<th>&gt; 25</th>
<th>&lt; 25</th>
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<tr>
<td>12 months post transplant</td>
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</tbody>
</table>

Augustine et al., AJT 2005
Specialized memory subsets: regionalization of immune surveillance

<table>
<thead>
<tr>
<th>Feature</th>
<th>Tissue Resident</th>
<th>Effector</th>
<th>Central</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Non-Lymphoid Tissues</td>
<td>Peripheral tissues (lung &amp; liver) and spleen</td>
<td>Lymphoid tissues</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lymph node and spleen</td>
</tr>
<tr>
<td>Cytokine</td>
<td>Immediate effector cytokines IFNγ, TNF</td>
<td>Immediate effector cytokines IFNγ, TNF</td>
<td>IL-2</td>
</tr>
<tr>
<td>Killing</td>
<td>immediate</td>
<td>immediate</td>
<td>inducible</td>
</tr>
<tr>
<td>Homing molecule</td>
<td>Sessile, non-circulating CD69+ CD103+</td>
<td>Peripheral homing CCR5 &amp; 6</td>
<td>Lymphoid homing CCR7, CD62L</td>
</tr>
<tr>
<td>Putative function</td>
<td>Immuno-surveillance in non-lymphoid tissues</td>
<td>Immediate recall at peripheral barrier sites</td>
<td>Sustained memory response</td>
</tr>
</tbody>
</table>
B Cell Differentiation Pathways

But what role do these play and can/should we assess?

This is what we measure
Detecting the Humoral Alloimmune Response: We Need More Than Serum Antibody Screening

Gonca E. Karahan, Frans H. J. Claas, and Sebastiaan Heidt

Abstract: Whereas many techniques exist to detect HLA antibodies in the sera of immunized individuals, assays to detect and quantify HLA-specific B cells are only just emerging. The need for such assays is becoming clear, as in some patients, HLA-specific memory B cells have been shown to be present in the absence of the accompanying serum HLA antibodies. Because HLA-specific B cells in the peripheral blood of immunized individuals are present at only a very low frequency, assays with high sensitivity are required. In this review, we discuss the currently available methods to detect and/or quantify HLA-specific B cells, as well as their promises and limitations. We also discuss scenarios in which quantification of HLA-specific B cells may be of additional value, besides classical serum HLA antibody detection.

(Transplantation 2015;99: 908-915)
HLA-Specific B Cells

1. A Method for Their Detection, Quantification, and Isolation Using HLA Tetramers

Andrea A. Zachary, Dessislava Kopchaliiska, Robert A. Montgomery, and Mary S. Leffell

(Transplantation 2007;83: 982–988)

![Graphs showing frequency of tetramer-positive B lymphocytes: subjects with antibody vs. those without.]

**Table 1.** Frequency of tetramer-positive B lymphocytes: subjects with antibody vs. those without

<table>
<thead>
<tr>
<th>Tetramer</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibody positive&lt;sup&gt;b&lt;/sup&gt;</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Antibody negative&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>27</td>
<td>4.07 (1.34)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>19</td>
<td>1.55 (0.86)</td>
<td>1×10&lt;sup&gt;-9&lt;/sup&gt;</td>
</tr>
<tr>
<td>A24</td>
<td>17</td>
<td>4.43 (1.39)</td>
<td>16</td>
<td>2.19 (1.39)</td>
<td>4.4×10&lt;sup&gt;-6&lt;/sup&gt;</td>
</tr>
<tr>
<td>B7</td>
<td>23</td>
<td>5.46 (1.90)</td>
<td>22</td>
<td>3.25 (1.50)</td>
<td>5.0×10&lt;sup&gt;-5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of subjects.

<sup>b</sup> Subjects with current or historic antibody specific for the tetramer HLA antigen.

<sup>c</sup> Subjects with no history of antibody to the tetramer HLA antigen.

<sup>d</sup> Frequencies are given as the percent of CD19<sup>+</sup> cells. Values in parentheses are standard deviations.
# HLA-Specific B Cells

## II. Application to Transplantation

*Andrea A. Zachary, Dessislava Kopchaliiska, Robert A. Montgomery, Joseph K. Melancon, and Mary S. Leffell*

*(Transplantation 2007;83: 989–994)*

## Table 2: Frequency of tetramer positive B lymphocytes among patients categorized by transplant history

<table>
<thead>
<tr>
<th>Tetramer</th>
<th>N</th>
<th>Antibody negative&lt;sup&gt;a&lt;/sup&gt;</th>
<th></th>
<th>Antibody positive&lt;sup&gt;b&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Previous transplant</td>
<td></td>
<td>Previous transplant</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td>No</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Yes</td>
<td></td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P</td>
<td></td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>19</td>
<td>1.18&lt;sup&gt;c&lt;/sup&gt; (0.66)</td>
<td>1.83 (0.92)</td>
<td>0.05</td>
<td>4.03 (1.46)</td>
</tr>
<tr>
<td>A24</td>
<td>16</td>
<td>1.98 (1.15)</td>
<td>2.47 (0.63)</td>
<td>0.15</td>
<td>4.34 (1.08)</td>
</tr>
<tr>
<td>B7</td>
<td>22</td>
<td>2.35 (1.09)</td>
<td>4.14 (1.38)</td>
<td>0.002</td>
<td>5.24 (1.83)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Patients lacking antibody specific for the tetramer.

<sup>b</sup> Patients with antibody specific for the tetramer.

<sup>c</sup> Frequency is the percentage of tetramer positive cells among CD19+ cells. Numbers in parentheses are standard deviations.
A Novel ELISPOT Assay to Quantify HLA-Specific B Cells in HLA-Immunized Individuals


Figure 3: B cells from two healthy individuals (left panel HLA class I typed A3, B7, B15, Cw3, Cw7 and right panel A3, A28, B44, B56, Cw1, Cw6, respectively), who were immunized against HLA-A2, but not HLA-A1 or HLA-A11, formed spots against HLA-A2 and no spots against HLA-A1 or HLA-A11. *: no HLA-specific spots detected.
**Table 1:** Concurrent determination of HLA-A2-specific B cells (by spot formation) and their secreted HLA antibody (by Luminex assay)

<table>
<thead>
<tr>
<th>Individual</th>
<th>Immunization status</th>
<th>Spot number(^1)</th>
<th>MFI supernatant(^2)</th>
<th>MFI neg. control</th>
</tr>
</thead>
<tbody>
<tr>
<td>70a</td>
<td>A2 immunized</td>
<td>99</td>
<td>59.79</td>
<td>0.98</td>
</tr>
<tr>
<td>71a</td>
<td>A2 immunized</td>
<td>0</td>
<td>0.00</td>
<td>0.76</td>
</tr>
<tr>
<td>71b</td>
<td>A2 immunized</td>
<td>6</td>
<td>61.80</td>
<td>0.75</td>
</tr>
<tr>
<td>73a</td>
<td>A2 immunized</td>
<td>8</td>
<td>112.18</td>
<td>1.48</td>
</tr>
<tr>
<td>73b</td>
<td>A2 immunized</td>
<td>24</td>
<td>140.22</td>
<td>1.00</td>
</tr>
<tr>
<td>70b</td>
<td>Nonimmunized</td>
<td>0</td>
<td>0.00</td>
<td>1.14</td>
</tr>
<tr>
<td>71c</td>
<td>Nonimmunized</td>
<td>1</td>
<td>0.00</td>
<td>2.91</td>
</tr>
<tr>
<td>71d</td>
<td>Nonimmunized</td>
<td>0</td>
<td>0.00</td>
<td>1.10</td>
</tr>
<tr>
<td>73d</td>
<td>Nonimmunized</td>
<td>0</td>
<td>3.90</td>
<td>0.90</td>
</tr>
</tbody>
</table>

\(^1\) Per million total B cells.

\(^2\) MFI positive control serum: 3012.75.
Detecting the Humoral Alloimmune Response: We Need More Than Serum Antibody Screening

Gonca E. Karahan,¹ Frans H. J. Claas,¹ and Sebastiaan Heidt¹

Abstract: Whereas many techniques exist to detect HLA antibodies in the sera of immunized individuals, assays to detect and quantify HLA-specific B cells are only just emerging. The need for such assays is becoming clear, as in some patients, HLA-specific memory B cells have been shown to be present in the absence of the accompanying serum HLA antibodies. Because HLA-specific B cells in the peripheral blood of immunized individuals are present at only a very low frequency, assays with high sensitivity are required. In this review, we discuss the currently available methods to detect and/or quantify HLA-specific B cells, as well as their promises and limitations. We also discuss scenarios in which quantification of HLA-specific B cells may be of additional value, besides classical serum HLA antibody detection.

(Transplantation 2015;99: 908–915)
A memory B cell crossmatch assay for quantification of donor-specific memory B cells in the peripheral blood of HLA-immunized individuals


Accepted manuscript online: 30 March 2017
It’s not as easy as it looks!

1) Peripheral blood (30 mL)
2) Ficoll-Hypaque isolation
3) B cell enrichment/T cell depletion
4) 7 day cell culture/stimulation-L-CD40L cells as stimulators plus IL-2, IL-10, IL-21 TLR-9 Ligand
5) Collection, freezing and storage of culture supernatant for ab testing.

Labor intensive, extensive QC, proficiency testing, maintenance of cell cultures, etc
Antibody Production is Controlled by the Balance of $T_{FH}$ and $T_{FR}$

Antibody Production is Controlled by the Balance of $T_{FH}$ and $T_{FR}$
Summary

• One test for all issues related to antibodies
• Not quantifiable
• Not uniform
• The tip of the iceberg
Conclusions

• Current tools are better than anything we’ve had before. But they remain rudimentary.

• Antibodies are surrogates for sensitization/memory. They tell only one part of a story.

• Risk assessment by antibody alone is at best incomplete, at most misleading.

• Need to transition to cellular assays for additional (better?) information

• Current testing for T and B cell memory still in early stages of development. Not yet quantifiable, labor intensive, clinical application still speculative.

• Moving forward-AUTOMATION. VETTING. CLINICAL UTILITY.

• Cannot reliably implement cell based assays without this consideration