HLA ANTIBODY ATTRIBUTES

Nicole M Valenzuela, PhD, D(ABHI)
UCLA Immunogenetics Center
Department of Pathology and Laboratory Medicine,
University of California, Los Angeles
Disclosure

I have no relevant financial disclosures.
Learning Objectives

1. Understand the basic diverse effector functions of antibodies and how they contribute to AMR

2. Understand the determinants controlling antibody effector functions

3. Learn how HLA antibody effector function and characteristics may be identified and what’s known to date about their clinical significance
So…there is much more to AMR and TV than complement!

HLA DSA \neq AMR \neq HLA DSA

Why is there stable graft function and/or normal histology in some patients with circulating DSA?

How are HLA antibodies causing these types of graft injury?
Human Immunoglobulin System

See Vidarsson Front Immunol 2014
Antibody-FcγR Functions

- Many cell types have Fc gamma receptors: Monocytes, NK cells, neutrophils, DCs, B cells
- Activation of innate immune cells
  - Natural killer (NK cells)
  - Monocytes
  - Neutrophils
- Phagocytosis and opsonization
  - Cooperate with complement receptors to engulf antigen
- Antibody-dependent cell mediated cytotoxicity by NK cells, possibly monocytes
  - Important for many depleting therapeutic antibodies
  - Not definitively shown for HLA antibodies against vascular cells
- Enhancement of adhesion to endothelium
  - Send signals to firm adhesion receptors (integrins)
Factors controlling antibody binding to C1q and Fc receptors

• Many of the same determinants control both C1q binding and Fc receptor binding:
  – Sequence (subclass)
  – Hinge region flexibility
  – Antibody abundance and density of antigen
  – Glycosylation

Diebolder Science 2014
# Affinity for FcγRs

<table>
<thead>
<tr>
<th>FcγRI</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++</td>
<td>-</td>
<td>++++</td>
<td>++</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FcγRIIa (mono/PMN)</th>
<th>+++</th>
<th>++/+*</th>
<th>++++</th>
<th>+</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>FcγRIIb (inhibitory)</th>
<th>+</th>
<th>-</th>
<th>++</th>
<th>+</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>FcγRIIIa (NK cells)</th>
<th>++++/+++*</th>
<th>+/−*</th>
<th>++++</th>
<th>++/−*</th>
</tr>
</thead>
</table>

* Depends on the allele of the Fc receptor

Adapted from Bruhns *Blood* 2009
Experimental Evidence: NK cells

- Reduced TV in NK cell-impaired mice with DSA \(^1, 2, 3, 4\)

- In murine allografts, there is rapid infiltration of NK cells and markers of activation that correlate with DSA \(^5\)

- Depletion of NK cells attenuates rejection \(^6\)

- In the absence of NK cells, DSA triggers an indolent and progressive injury \(^5\)

---

1 Hirohashi AJT 2011
2 Zhang Transpl 2014
3 Uehara JI 2005
4 Lin AJT 2016
5 Yagisawa Kid Int 2019
6 Kohei Kid Int 2016
Experimental Evidence: NK cells

• Possible mechanisms:
  – Non-self recognition
  – IFNγ-dependent
  – perforin/ADCC & Fas/FasL dependent killing

• Lin AJT 2016, Kohei Kid Int 2016

Raj Front Immunol 2016
Thomas Trends Mol Med 2014
Clinical Evidence: NK cells

NK cell markers are observed within rejecting allografts\(^2, 3\)

- In renal transplant biopsies, NK cell-associated transcripts are increased in grafts with AMR\(^4\)
- In cardiac transplant recipients, FCGR3A genotype was associated with risk of CAV\(^1\)
- Renal transplant recipients with higher “NK-CHAT” allo-reactivity associated with C4d staining\(^5\)
  - Patients showed variability in their \textit{in vitro} rituximab response, which has been well-described in oncology

\(^1\) Paul \textit{Circ} 2018
\(^2\) Javaheri \textit{Transpl Immunol} 2018
\(^3\) Yazdani \textit{Kid Int} 2019
\(^4\) Parkes \textit{Transpl} 2017
\(^5\) Legris \textit{Front Immunol} 2017
Does eliminating Fc functions (complement and FcγR) prevent graft injury?
Antibody-FcγR Functions: Therapeutic Strategies

- IgG-modifying enzymes
  - IdeS
  - EndoS

- Notable take-homes from IdeS clinical trial outcomes:
  - One hyperacute rejection (thought to be due to IgM/A or AECA)
  - Needed B cell depletion therapy
  - Antibodies reconstituted/rebounded
  - Some patients still experienced clinical and subclinical rejection (similar to Eculizumab)

Jordan NEJM 2017
NCT02224820, NCT02426684, NCT02475551
HLA Antibody-Induced Signaling: Experimental (\textit{in vitro}) Evidence

AGONISTIC SIGNALING IN VASCULAR ENDOTHELIUM AND SMOOTH MUSCLE

- Analogous to reverse signaling in APCs at the immunologic synapse
- Tyrosine kinase signaling cascades leading to ERK, mTOR, S6RP and S6K activation
- Pro-survival signaling at low concentrations (Bcl-2, Bcl-XL)
- Rapid calcium-dependent mobilization of vesicles, release of vWF and P-selectin
- Increased production of chemokines and cytokines
- Increased production of MMPs

FUNCTIONAL CONSEQUENCE

- Pro-growth and increased proliferation
- Resistance to cell death and complement-mediated injury
- Increased adhesion of neutrophils, platelets and monocytes
- Activation of T cells and Th17 differentiation
- Increased tissue remodeling?

See; Thomas \textit{Trends Mol Med} 2015 for review
Transplant Vasculopathy

Kidney transplant arteriopathy (J. Zuckerman)

Heart: Nearly occluded artery (G. Fishbein)

Liver
Naini Practical Atlas of Transplant Pathology

Lung
Wallace Practical Atlas of Transplant Pathology

Bowel
Koo Practical Atlas of Transplant Pathology

VCA
Smart Practical Atlas of Transplant Pathology

Pancreas
Swanson Practical Atlas of Transplant Pathology

normal
Molecular Signatures of AMR

- Transcripts within cardiac and renal biopsies with AMR\textsuperscript{1,2}
  - Increased endothelial-specific signatures (ENDAT)
  - Increased NK cell-associated transcripts
  - IFNγ signatures
  - Monocyte/macrophage also increased across rejection
  - AMR gene scores increased with pAMR severity and were more highly associated with MVI than C4d only in pAMR\textsuperscript{1}

\textsuperscript{1} Venner AJT 2015
\textsuperscript{2} Loupy Circ 2017
\textsuperscript{3} Afzali AJT 2016
Capillary phosphorylation of mTOR targets (S6K, S6RP) are significantly associated with AMR. Recipients on mTORi have significantly reduced incidence of CAV compared with CNI-based regimens.

| Table 3 |
| Association between grades of staining of S6K, S6RP, ERK and pAMR |
| Odds Ratio | p-value | 95% CI |
| S6K, grade 0 | Baseline | N/A | N/A |
| S6K, grade 1 | 18 | 0.001 | 3 – 100 |
| S6K, grade 2 | 52 | 0.001 | 6 – 425 |
| S6K, grade 3+ | 49 | 0.001 | 5 – 521 |
| S6RP, grade 0 | Baseline | N/A | N/A |
| S6RP, grade 1 | 4 | 0.06 | 1 – 13 |
| S6RP, grades 2, 3+ | 10 | 0.008 | 2 – 52 |
| ERK, grade 0 | Baseline | N/A | N/A |
| ERK, grade 1 | 5 | 0.2 | 0.4 – 53 |
| ERK, grade 2 | 0.8 | 0.8 | 0.1 – 7 |
| ERK, grade 3+ | 0.4 | 0.4 | 0.04 – 4 |

1 Lepin AJT 2006
2 Li JHLT 2016
3 Tible JHLT 2013
4 Jennings Int J Cardiol 2018
# HLA Antibody Attributes

<table>
<thead>
<tr>
<th>Attribute</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activating FcR on mono/PMN/NK cells</td>
<td>better</td>
<td>worst</td>
<td>best</td>
<td>good</td>
</tr>
<tr>
<td>Inhibitory FcR</td>
<td>better</td>
<td>worst</td>
<td>best</td>
<td>good</td>
</tr>
<tr>
<td>Complement</td>
<td>better</td>
<td>good</td>
<td>best</td>
<td>worst</td>
</tr>
<tr>
<td>Agonistic Signaling</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>
Can we assess biological function [potential] with an *in vitro* diagnostic assay?
Subclass Assay: Purpose and Method

- **Purpose**: to characterize the IgG subclass (IgG1-4) of anti-HLA antibodies
- **Method**: Modified single antigen assay with swapped out secondary detection reagents against each subclass
- **Potential Utility**: To predict the biological activity and pathological potential of an antibody
IgG Subclasses of HLA antibodies

- Pre-transplant, anti-HLA antibodies are predominantly a mix of subclasses
- Pregnancy and transplantation stimulate a mix, while transfusion stimulates predominantly IgG1 only
- Pre-formed IgG1 or IgG4 DSA independently predictive of rejection in the first 30 days
- Only 1 patient showed IgG4 without IgG1
  - Reiterates that usually a mix of subclasses
IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury

Carmen Lefaucheur,*† Denis Viglietti,*† Carol Bentlejewski,‡ Jean-Paul Duong van Huyen,†§ Dewi Verneroy,* Olivier Aubert,† Jérôme Verine,§ Xavier Jouven,† Christophe Legendre,** Denis Glotz,* Alexandre Loupy,*† and Adriana Zeevi†

Figure 2. Identification of the three distinct rejection phenotypes according to the characteristics of the dominant donor-specific anti-HLA antibody (MFI, HLA class specificity, C1q-binding capacity, and IgG1–4).

IgG1, IgG2 C1q binding distinguish AMR vs. no AMR  
IgG3 distinguishes acute vs. subclinical  
IgG4 distinguishes acute vs. subclinical

IgG4 DSA is not associating with no rejection!
Subclass Assay: Pros and Cons

Pros
• More information than C1q or C3d
• Can infer more than complement!
  – Informs on other effector functions such as Fc receptor binding

Cons
• Not commercial/not well-validated
• Requires 5 total single antigen tests (expensive and laborious)
• Cannot directly compare the relative signals for each subclass
  – We don’t know the actual quantities and relative abundance of subclasses against HLA
• Some cross-reactivity of the secondaries (not a clean test)
• Some inability (~15%) to detect any subclasses even when total IgG signal was strong
  – Sensitivity?
• Recent trend in the literature to measure only IgG3 associations with outcome
  – Not enough comprehensive, reliable assessments have been reported to neglect the other subclasses
Time-dependent IgG subclass production

- Antigen properties and immune context shape the CSR response
- B cells that class switch cannot switch back
- Production of downstream constant regions suggests repeated antigen stimulation and germinal center reactions
- IgG3 or IgG1 suggests an early, recent immune response
- IgG4 points to prolonged antigen exposure
Implications for AMR Mechanistic Understanding and Therapy

• Understanding the “attributes” of an antibody might predict pathogenic functions
  – Remains to be demonstrated experimentally

• Antibody subclasses are generated under different conditions and times
  – Not only important for effector functions;
  – Implications for biology of the immune response
    • ‘Imbalances’ in IgG subclasses are pathogenic in or markers of many diseases
  – And types of B cells producing those antibodies

• May have utility describing the individual’s alloimmune status (memory, newly activated, chronically stimulated)
  – Could it also point to potential efficacy (or inefficacy) of different B cell targeted therapies?
In closing...

ALL HLA antibodies that can bind donor cells, regardless of subclass, are likely to be pathogenic [until proven otherwise]

Stratified risk:
- cytotoxicity ~ hyperacute or accelerated rejection
- innate immune activation ~ acute rejection, TV
- vascular signaling ~ subclinical, smoldering, TV?

Beyond complement and FcγRs:
- Agonistic signaling
- Association with chronic antigen exposure
- Association with subclinical AMR
Questions for Discussion

• Is there clinical utility in further characterizing HLA antibody attributes?

• Role for recipient polymorphisms?
  – Complement
  – FcγRs
  – Immunoglobulin allotypes (ex. FcRn affinities)
  – Implications for risk stratification and appropriate therapies?

• How will we integrate all the information from these myriad tests into a refined, accurate and personalized approach for our patients?
Mechanisms and histological features of (HLA) antibody mediated injury

In vitro detection of complement binding or activation by HLA antibodies (C1q, C3d, CDC-XM) is not directly analogous to actual human complement activation in vivo.

IgG1 is most abundant in the serum and dominates most immune responses. Most immune responses elicit a mixture of IgG subclasses.

IgG4 is the only true “non-complement fixing” subclass. But it was still associated with rejection.
Determinants of C1q Binding in the Single Antigen Bead Assay

Stefan Schaub,1 Gideon Hönger,1 Michael T. Koller,2 Robert Liwski,3,4 and Patrizia Amico1

**FIGURE 2.** Correlation of standard C1q MFI with IgG subclass MFI. MFI values are plotted on a log scale. Only IgGpan+/IgGsubclass+ SAB were included (n=2,665). Individual SAB are color coded by the percentage of strong C-binding subclasses. The given $r^2$ were calculated by simple logistic regression using the standard C1q MFI greater than 300 cutoff.
Complement is not necessarily required for AMR or transplant vasculopathy

Evident both from murine studies and from clinical experience

- C3 deficient recipient mice develop transplant arteriopathy in the presence of DSA
- …although it may enhance TV and adaptive alloreactivity in general
- C4d negative AMR recognized in renal and heart transplantation
  - Little benefit to prophylactic terminal complement inhibition in DSA+ renal transplant patients with deteriorating graft function

1 Hirohashi AJT 2010
2 Jane-Wit Circ 2013
3 Qin AJT 2016
4 Haas AJT 2014
5 Berry JHLT 2013
6 Kulkarni AJT 2016
It’s the patients who experience rejection that have the worst long-term outcomes.

Kidney: Sicard JASN 2015

Kidney: Lefaucheur JASN 2010

Heart: Lefaucheur JASN 2015

Liver: O’Leary Transpl 2017

Lung: DeNicola JHLT 2013

Lung: Witt JHLT 2013
Human IgG Subclasses

<table>
<thead>
<tr>
<th></th>
<th>IgG3</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abundance in circulation</td>
<td>Low ~4%</td>
<td>Highest ~50-60%</td>
<td>High ~30-50%</td>
<td>Low ~4%</td>
</tr>
<tr>
<td>Half-Life</td>
<td>7-21 days*</td>
<td>21 days</td>
<td>21 days</td>
<td>21 days</td>
</tr>
<tr>
<td>Affinity for Antigen</td>
<td>Relatively lowest</td>
<td>High</td>
<td>High</td>
<td>Highest</td>
</tr>
<tr>
<td>Notable for</td>
<td>Long hinge region</td>
<td>Nearly always present and dominant</td>
<td>Response to carbohydrate as well as protein antigens</td>
<td>Ability to form monovalent arms and bispecific heterodimers</td>
</tr>
<tr>
<td>Tempo</td>
<td>Earliest, transient</td>
<td>Early and memory</td>
<td>Later</td>
<td>Much later, chronic antigen exposure</td>
</tr>
</tbody>
</table>
Human Immunoglobulin System

**Isotypes**
- IgD
- IgM
- IgG
- IgA
- IgE

**Subclasses**
- IgG1
- IgG2
- IgG3
- IgG4
- IgA1
- IgA2

**Allotypes**
- G1m
- G2m
- G3m
- A2m

<table>
<thead>
<tr>
<th>Subclass</th>
<th>Allotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>G1m 1(a)</td>
</tr>
<tr>
<td></td>
<td>G2m 23(n)</td>
</tr>
<tr>
<td>IgG2</td>
<td>G3m g1</td>
</tr>
<tr>
<td>IgG3</td>
<td>g5</td>
</tr>
<tr>
<td>IgG4</td>
<td>b0</td>
</tr>
<tr>
<td>IgA1</td>
<td>b1</td>
</tr>
<tr>
<td>IgA2</td>
<td>b3</td>
</tr>
</tbody>
</table>

... 13 alleles

Exist in haplotypes differentially distributed among ethnic groups
Methods and Approach

Variable regions of murine anti-HLA class I (W6/32)

Chimeric human-mouse pan HLA I IgG expressed in CHO cells

Constant regions of human IgG (S Morrison)

Same antigen binding region on different subclasses

Recognizes monomorphic epitope on all HLA class I molecules → recognize antigen on all beads with the same affinity

While less potent than IgG1, IgG2 was still capable of fixing C1q on single antigen beads at high concentrations
HLA I IgG1 and IgG2 both trigger cytotoxicity in the CDC assay

Rabbit C’

Fig. 3. Fixation of human (○), guinea pig (△), and rabbit (□) complement by chimeric anti-DNS IgG antibodies. Mouse IgG isotypes (A) and human isotypes (B) were tested in complement consumption assays (see Materials and methods). Each data point represents the mean of four to eight measurements.
Monocyte adherence is enhanced by complement.

Complement augments endothelial cell activation and monocyte adherence in the presence of HLA antibodies.