Therapeutic and Management Strategies for AMR:
A UCLA Cross-Disciplinary Workshop to improve diagnosis and treatment of Acute and Chronic AMR

Friday, Nov. 8, from 12 - 5 p.m.
Tamkin Auditorium, B-130 / Ronald Reagan UCLA Medical Center
1. Treating/Reversing AMR

2. Prevention of pre-transplant Sensitization

3. Prevention of sensitization at the time of transplant

4. Monitoring for DSA and/or “early ABMR”

5. Protocol Biopsies
AMR Workshop
How do we Diagnose AMR?

Workshop Summary and Next Steps
### Post-Transplant DSA Assessment

<table>
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<tr>
<th></th>
<th>DSA-</th>
<th>DSA+</th>
<th>Desensitization</th>
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<tr>
<td>All Patients</td>
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<td>Adult Renal</td>
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<tr>
<td>DSA+</td>
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<td>4-5d</td>
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All patients with suspected AMR
Future Directions

- Establish transplant biorepository
- Establish transplant data repository- XDR
  - UCBRAID infrastructure
- Protocolize frequency of DSA testing across all organs and correlate with graft pathology
  - Consider time points
  - Assess complement vs. non-complement Ig isotypes
  - Non-HLA antibodies
- Determine the characteristics of AMR across all organs- are there similarities? What are the differences?
  - Apply New technologies & Biomarkers
    - Genomics,
    - Phosphoproteomics and proteomics
    - Immunogenetic factors: FcR polymorphisms
    - Immune assessment (immunophenotyping, direct/indirect allore cognition)
- Development measures of effective/ineffective immunosuppression
  - Responders vs. non-responders
  - Immune Memory
  - Immune senecence/exhaustion
- Transplant seminar series
- Proceedings of the AMR WS
- Planning Grant
UCLA PEDIATRIC RENAL TRANSPLANTATION
APPROACH TO ABMR

• Prevention of sensitization- Pre Transplant considerations
  • Avoid Transfusions? – **Yes**
  • Avoid Pre-Tx DSA > 3000 MFI – **Yes**
  • *Try* to avoid removing transplant – **Yes**

• Prevention of sensitization – Post Transplant considerations
  • Optimal HLA match - **No**
  • Optimize Adherence - **Yes [but how??]**
  • Selection of Immunosuppression regimen
    • Sirolimus / Everolimus - **??**
    • Belatacept - **?? [no post-rejection DSAs]**

• Monitoring for DSA
  • Periodic assessment of DSA – **Yes**
  • Does de novo DSA → biopsy – **Yes [any positive level]**
  • If biopsy is negative for ABMR, treat ? – **Yes, IVIG (see next slide)**

• Protocol Biopsies – **Yes [6, 12 & 24 months]**
TREATMENT AND MANAGEMENT OF ABMR

- **Established ABMR**
  - **C4d+ or DSA+ & histological confirmation**
    - Sucrose free IVIG 2 g/kg; repeat in 1 month
    - Rituxan 375/m² x 1
    - Rebiopsy in 6-8 weeks
  - **C4d – but histological changes ± DSA**
    - Same as above
  - **C4d+ only (no DSA or histological changes)**
    - IVIG as above but no Rituxan
    - Re-biopsy
    - Check for non-HLA antibody

- **DSA+ only**
  - MFI <5000 : follow clinically
  - MFI >5000 : IVIG as above
  - If no response or increase - Rebiopsy
DESENSITIZATION

- Protocol
- Pre-transplant:
  - IVIG 2g/kg Month 1, 3, 4, 5
  - Rituxan 375 mg/m2/dose month 2
  - Get baseline DSAs, anti-endothelial antibody, MICA
  - Monitor monthly antibodies for efficacy and review at DSA meeting monthly with Immunogenetics to potentially take off unacceptable antigens
- Post-transplant:
  - Thymoglobulin induction
  - Redose IVIG 2g/kg, check B cell subsets may need redose Rituxan, monitor antibodies
  - Biopsy if possible and consider plasmapheresis, Bortzemib or Ecluzimib if compromised kidney function and antibodies
OUR EXPERIENCE WITH BORTEZIMIB

• 6 pediatric patients were treated with Bortezimib for refractory AMR with DSA’s
• Patient’s Age Range : 14 to 19 years
• 1 female and 5 males
• Developed AMR anywhere between 1 year to 10 years post transplant
• 3 of these patients were known to be non compliant
• All patient’s were maintained on immunosuppression that included tacrolimus, cellcept and prednisone
POST TX PROTEASOME INHIBITION PROTOCOL

- Day 1: plasmapheresis #1, Bortezomib #1, 100 mg Solumedrol
- Day 4: plasmapheresis #2, Bortezomib #2, 100 mg Solumedrol
- Day 7: plasmapheresis #3, Bortezomib #3, 50 mg solumedrol
- Day 10: plasmapheresis #4, Bortezomib #4, 50 mg solumedrol
- Day 14: plasmapheresis #5
- Day 15: plasmapheresis #6
- Day 16: plasmapheresis #7, IVIG
- Bortezomib is 1.3 mg/m2, given w/ Solumedrol, Benadryl, & Tylenol and Zofran
- Ideally Rituxan should also be administered on Day 1, after Bortezomib
NON COMPLIANT PATIENTS

• Developed AMR with DSA’s earlier: As early as 1 year post transplant
• All the patient’s had C4d positive AMR on biopsy prior to initiation of Bortezomib with +DSA’s (Mean MFI was 15,000)
• All 3 patient’s had no change in their biopsies or DSA’s 2 months after bortezomib.
• 1 patient had a decrease in creatinine from 3.3 to 1.2
COMPLIANT PATIENTS

- Developed AMR later
- All of them had C4d+ AMR on their biopsies and only 1 patient had a change post bortezomib to C4d negative AMR
- Change in creatinine was not significant
- No significant changes was noted in their DSA’s pre and post bortezomib
OUTCOMES

• Only 1 patient lost her graft
• No adverse events were noted secondary to administration of Bortezimib
• Patient’s received anywhere between 1 to 3 rounds of Bortezimib
• All of these were late ABMR
MORE QUESTIONS THAN ANSWERS
Therapeutic and Management Strategies for AMR:
A UCLA Cross-Disciplinary Workshop to improve diagnosis and treatment of Acute and Chronic AMR

Mario C Deng MD FACC FESC
Professor of Medicine & Medical Director
Advanced Heart Failure/Mechanical Support/Heart Transplantation
Ronald Reagan Medical Center
Division of Cardiology
Department of Medicine
David Geffen School of Medicine at UCLA
University of California, Los Angeles
USA
# UCLA HTx surveillance protocol 7/1/11

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*single-antigen-based antibody testing (or flow crossmatch) in suspected rejection/graft dysfunction (frequency determined by clinical suspicion)
**Bx including C4D/CD68 (do also single-antigen-based antibody testing & cyclex)
***AlloMap (do also single-antigen-based antibody testing & cyclex)
**** earliest timepoint 2 mo (day 56) post transplant if clinician & patient feel comfortable, Echo preferentially included
***** if LO REJECTION RISK, if HI REJECTION RISK BX until stabilization, if INTERMEDIATE RISK> alternate BX and ALLOMAP
****** DSE/radionuclide and LHC/IVUS alternating years if no CAV and clinician & patient comfortable
1. Induction immunosuppression will not be administered to all heart transplant recipients.

2. Induction therapy may be considered in adult heart transplant candidates with elevated panel reactive antibodies (PRA) $>20\%$, history of mechanical circulatory support device, prior transplantation, and/or are multiparous females.

3. Treatment of rejection should be provided for adult heart transplant recipients who have biopsy-proven acute cellular or antibody-mediated rejection or demonstrate hemodynamic compromise.

UCLA Health Transplant Services 2013 - Policy & Procedure for Adult Heart Transplant: Induction & Rejection Therapy
4. The Transplant Cardiologist, Cardiothoracic surgeon, Transplant Pharmacist, along with relevant clinical staff (Herein called the ‘Transplant Team’) shall choose the appropriate induction/rejection regimen tailored to the individual patient needs with the aim of minimizing both the risk of future rejection and infection. Individual patient and donor characteristics, as well as potential immunosuppressive therapy toxicities will be taken into consideration when choosing a regimen.
5. The judgment of an experienced Transplant Team is essential in choosing the most appropriate induction/rejection immunosuppression regimen for a given patient. The guidelines set forth in this Policy (& Procedure) are not meant to substitute for good clinical judgment.

6. Patients who undergo orthotopic heart transplant at the University of California, Los Angeles, will be given the option of participating in any clinical trials for which they are eligible. The guidelines provided in this document shall only be used for patients ineligible or unwilling to participate in available trials or in trials where specific therapy procedures are not delineated.
1.1 Hemodynamic Compromise:
1.1.1 Cardiac Index < 2.0
1.1.2 Clinical heart failure
1.1.3 LVEF < 40% with need for inotropic support
1.2 Antibody mediated rejection: diagnosis by histology
1.3 All biopsies graded by 2005 ISHLT Nomenclature
1. Patients with elevated panel reactive antibodies (PRA) >20%, history of mechanical circulatory support device, prior transplantation, and/or multiparous females may be considered for desensitization therapy.

2. The Transplant Cardiologist, Cardiothoracic surgeon, Transplant Pharmacist, along with relevant clinical staff (Herein called the ‘Transplant Team’) shall choose the appropriate desensitization regimen tailored to the individual patient needs with the aim of minimizing both the risk of future rejection and infection.

DEFINITIONS: Sensitized Patient: panel-reactive HLA antibodies (PRA) over 10%, Highly Sensitized Patient: panel-reactive HLA antibodies (PRA) over 80%
1. Plasmapheresis and Immune Globulin Therapy
   1.1 Plasmapheresis (daily to every other day for 5 sessions)
   1.2 Sucrose-free immune globulin (IVIG) 1 g/kg/day for two days

2.1 Day -5 to Day -0: Plasmapheresis
2.2 Day 0 and Day 30: IVIG 1g/kg x 2 days
2.3 Day 7 and Day 22: Rituximab 375mg/m2 or 1000mg

3.1 Plasmapheresis prior to bortezomib on day 1, 4, 7/8, 10/11, AND daily x 3 >72 hours after last bortezomib dose (Day 13/14)
3.2 Bortezomib 0.7-1.3mg/m2 IV/Subcut on day 1, 4, 7/8, 10/11

UCLA Health, Transplant Services 2013 - Policy & Procedure for Adult Heart Transplant: Desensitization Therapy
3.1.1 pAMR1: Pursue treatment if evidence of graft dysfunction/hemodynamic compromise

3.1.2 pAMR2: Pursue treatment if evidence of graft dysfunction or presence of DSA

3.1.3 pAMR3: Pursue treatment in absence of contraindications, unless patient is asymptomatic without presence of donor-specific antibodies or graft dysfunction

3.1.4 Treatment of AMR should be pursued regardless of AMR grade if there is evidence of graft dysfunction or hemodynamic compromise.

3.1.5 One or more therapy options may be required per Adult Heart Transplant Team.

UCLA Health Transplant Services 2013 - Policy & Procedure for Adult Heart Transplant: Induction & Rejection Therapy
3.2.1 Methylprednisolone 500mg IV Qday x 3 days
3.2.2 Plasmapheresis daily x 5 days
3.2.2.1 Alert Hemapheresis Team at x47177 immediately upon identification of potential need for plasmapheresis
3.2.2.2 Plasmapheresis should be initiated within 24 hours, based upon severity of rejection, with more urgent cases taking priority
3.2.2.3 Asymptomatic AMR patients may receive plasmapheresis daily or every other day to accommodate daily plasmapheresis in patients with hemodynamic compromise
4.1 Patients who have completed de novo antimicrobial prophylaxis who then undergo cytolytic or high-dose steroid therapy for rejection should be reinitiated on CMV and pneumocystis prophylaxis for a minimum of six (6) months following completion of rejection therapy. Refer to Adult Heart Transplant Antimicrobial Prophylaxis Policy & Procedure
UCLA Htx 1 year Survival

Log rank
\[ p = 0.931 \]
AMR Workshop: Treatment Lung Transplantation

David J. Ross, MD
Medical Director, Lung & Heart-Lung Transplant Program; Director, Pulmonary Hypertension & Thromboendarterectomy Program
Professor of Medicine, David Geffen School of Medicine
Division of Pulmonary, Critical Care, Allergy & Immunology
Ronald Reagan – UCLA Medical Center
Treating / Reversing AMR: Lung Transplant

- IVIG total 2.0 $gm/kg$ + Plasmapheresis /exchange x 5 sessions + Rituximab 375 $mg/m^2$ weekly
- +/- Eculizumab
- +/- Rabbit Anti-thymocyte Globulin (RATG) x 7 day course.

David Ross; UCLA Lung & Heart-Lung Transplant; 11/2013
Prevention of pre-transplant Sensitization: Lung Transplant

- Limitation of pre-sensitization events: attempt to avoid PRBC or platelet transfusion.
- “Surveillance” for potential allosensitization after respiratory viral infections with repeated HLA Single Antigen monitoring (*monthly x 3*).
- HLA “matching” with donor **not** feasible with lung transplant due to paucity of donor availability.

David Ross; UCLA Lung & Heart-Lung Transplant; 11/2013
Prevention of sensitization at time of Transplant: Lung Transplant

- HLA “matching” with donor, *not* feasible with lung transplantation (*see above*)
- Discussion of alternative “induction” cytolytic (“standard protocol”: RATG or Basiliximab) e.g. Campath-1H but potentially increased risk infection post-lung transplant.
- IVIG + Rixumab treatment if DSA HLA Class I or II *pre-transplant* (MFI<5000).
Monitoring for DSA post-transplant: Lung Transplant

- “Single Antigen HLA Class I & II” POD #1, 7, 14 then monthly x 3 then Q 3-4 mos.
- De novo DSA (MFI>5000) Bronch + TBBx otherwise “surveillance” TBBx at weeks 1, 4-6, 12, 24.
- Detection “asymptomatic” DSA HLA Class I or II (MFI>100) treatment: IVIG x 3 mos. + Rituximab.
- (+) TBBx for ABMR treatment based on “clinical-radiologic-physiologic assessment” IVIG +/- Rituximab +/- Plasmapheresis/exchange +/- Eculizumab +/- RATG
Protocol (i.e. “Surveillance”) TBBx + BAL:
Lung Transplant

- **Weeks 1, 4-6, 12, 24 (BAL only at Week 1 unless “clinical indication”)**
- “Collaborative Basic Science research” (e.g. Chemokine /Cytokines) routine on all BAL specimens *with HSPC Consent*
- “Respiratory Viral PCR” (Luminex™) on all BALF specimens + “routine” microbiologic studies.
“Investigational” for AMR: Lung Transplant

• U01 Multi-Center CTOT Grant Submission:
  (combined with University of Pittsburgh; PI: John McDyer, MD):
  – “Preemptive Proteasome inhibitor therapy for Donor specific Alloreactivity in Kidney & Lung Transplant Recipients”

David Ross; UCLA Lung & Heart-Lung Transplant; 11/2013
AMR Therapy in Intestine Transplantation

Laura J. Wozniak, MD, MS
UCLA, David Geffen School of Medicine
Pediatric Gastroenterology, Hepatology, & Nutrition

Douglas G. Farmer, MD
Department of Surgery
1. Treating/Reversing AMR

- Plasmapheresis
  - Access limitations
- High-dose IVIG
  - 1g/kg/day x 2days
  - Repeated every 2-4 weeks
- Rituximab
  - 375mg/m²
  - 1-4 weekly doses
- Bortezomib
- Eculizumab
2. Prevention of Pre-Transplant Sensitization

- Minimization of transfusion exposure
- Screen for Preformed anti-HLA Antibodies
- Treatment of highly sensitized candidate
  - Plasmapheresis (access limitations)
  - High dose IVIG
  - Borezomib
3. Prevention of Sensitization at the Time of Transplant

• None
4. Monitoring for DSA or “early AMR”

- No routine monitoring of DSA
  - Screening PRAs sent in re-transplant recipients
- Testing only occurs with atypical rejection or rejection resistant to standard therapies
AMR Therapy in Adult Liver Transplantation

Laura J. Wozniak, MD, MS
UCLA, David Geffen School of Medicine
Pediatric Gastroenterology, Hepatology, & Nutrition

Douglas G. Farmer, MD
Department of Surgery
1. Treating/Reversing AMR

- Plasmapheresis
- High-dose IVIG
  - 1g/kg/day x 2 days
  - Repeated every 2-4 weeks
- Rituximab
  - 375mg/m²
  - 1-4 weekly doses
- Antecedent rejection therapies
  - methylprednisolone pulse
  - Thymoglobulin
2. Prevention of Pre-Transplant Sensitization

• None
3. Prevention of Sensitization at the Time of Transplant

• None
4. Monitoring for DSA or “early AMR”

- No routine monitoring of DSA
  - Screening PRAs sent in re-transplant recipients
- Testing only occurs with atypical rejection or rejection resistant to standard therapies
5. Protocol Biopsies

• Not currently performed at our center in the setting of normal allograft function
5. Protocol Biopsies

- Weekly x 6-8
- Monthly x 6-12
- Quarterly to yearly or PRN thereafter
- Pathology
  - Routine H&E
  - CD4 not established at our center
  - Histopath of antibody mediated rejection ill defined
Treatment of AMR in Adult Kidney Transplant

Gerry Lipshutz, MD
Mike Bunnapradist, MD
We know AMR exists and can lead to graft loss
But what should we do AMR

• Treat them with IVIG, augmentation of immunosuppression? Soliris, Velcade, Rituxan, plasmapheresis?
DSA with mild graft dysfunction

• Mild dysfunction
  – IVIG 2 grams/Kg q month times 2
  – After IVIG, We tend to follow up DSA but we usually wont treat unless graft dysfunction
DSA with severe graft dysfunction

• Plasmapheresis and IVIG
• Resistant cases
  – Velcade
• Repeat DSA in 2 weeks or earlier, repeat above until recovery of renal function or when renal dysfunction is stable
DSA with good graft function

• We don’t know what to do
  – we don’t look for them we don’t do protocol biopsy
DSA with chronic changes

- Defined by evidence of CTG or significant chronic changes or overt proteinuria
  - Usually we won't treat
DSA Status in UCLA Heart Transplant Recipients

**Adult and Pediatric Heart**

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<th>3m</th>
<th>4m</th>
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<th>6m</th>
<th>8m</th>
<th>10m</th>
<th>12m</th>
<th>Quarterly</th>
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166 Heart transplant recipients
(Jan 1, 2010 to present)

23% de novo

32% with positive DSA post-TX
(53/166)

22 pre-TX DSA

7 Neg

11 persistent

4 persistent and De novo

3 class I

23 class II

12 class I/II

38 post-TX DSA

105 no Ab

1 need donor DP typing

68% persistent
DSA Status in UCLA Adult Heart Transplant Recipients

122 Adult Heart transplant recipients (Jan 1, 2010 to present)

21% de novo

20.5% with positive DSA post-TX (35/122)

- 14 pre-TX DSA
- 5 Neg
- 6 persistent
- 3 persistent and De novo
- 2 class I
- 16 class II
- 8 class I/II

64% persistent

81 no Ab

1 need donor DP typing
DSA Status in UCLA Pediatric Heart Transplant Recipients

44 Pediatric Heart transplant recipients (Jan 1, 2010 to present)

8 pre-TX DSA

43% with positive DSA post-TX (19/44)

2 Neg

5 persistent

1 persistent and De novo

75% persistent

12 post-TX DSA

27% de novo

1 class I

7 class II

4 class I/II

24 no Ab
Time to de novo DSA

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<tr>
<td>Class II</td>
<td>10 month</td>
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<td>Class I/II</td>
<td>6 month</td>
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Accumulative %

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<td>26%</td>
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<td>1-3 m</td>
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<td>71%</td>
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<td>12-18 m</td>
<td>8 patients</td>
<td>92%</td>
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Non-responder to IVIG

TX date: 4/17/03
First post-TX SAB: 2/6/06 DSA to DQ6 and DR51

IVIGX2
2/15/09

IVIG

TX
Non-responder to IVIG

First post-TX de novo DSA detected right after TX

First detected de novo DSA

IVIG

TX

IVIG
Responder to IVIG

First detected do novo DSA

IVIG

TX

B57

B45

A30

A66

DQ7

First detected do novo DSA
FcγRIIa Alleles Influence Monocyte Recruitment by HLA I Antibody-Activated Endothelial Cells

Nicole Valenzuela and Elaine Reed
Department of Pathology and Laboratory Medicine
UCLA Immunogenetics Center
Disclosures

• None to declare
Background

Intragraft CD68+ macrophage infiltrates:

• are characteristic of cardiac AMR (Fishbein *Hum Immun* 2012; Fedrigo *JHLT* 2013)
• exhibit pathogenic functions in experimental models of allograft rejection (Wu *Circ Cardiovasc Imaging* 2013; Liu *JI* 2012; Qi *Transpl* 2008)
• can act as effectors of direct tissue damage (Jose *Transpl* 2003) and present antigen to T cells (Horne *JI* 2008)
• confer worse outcome in patients when found in renal transplant biopsies (dos Santos *Ren Fail* 2013; Tinckham *Kidney Int* 2005)
Background

• HLA I antibodies stimulate endothelial exocytosis and P-selectin presentation

• P-selectin is necessary and sufficient for increased adherence of monocytes in vitro

• Blockade of P-selectin reduces macrophage infiltration into murine cardiac allograft during AMR
Background

- Monocytes express FcγRI (CD64) and FcγRIIa (CD32a)

- Human FcγRIIa is dimorphic
  - H131: $K_a = 4.5 \times 10^5$
    “high affinity”
  - R131: $K_a = 8 \times 10^4$

- Alleles are associated with autoimmunity, infection, response to antibody therapeutics, and allograft rejection
Hypothesis

- The various subclasses of HLA I antibodies will differentially promote monocyte recruitment due to affinity for FcγRs.

- Recipients carrying different alleles of FcγRIIa will exhibit different recruitment of monocytes during AMR.
Experimental Approach

- The variable regions of mouse anti-HLA I (W6/32) were cloned onto human constant regions to generate chimerized HLA I hIgG1 and hIgG2

- Human aortic endothelial cells were stimulated with chimeric HLA I hIgG1 and hIgG2

- Measured endothelial cell activation (P-selectin cell-based ELISA)

- Compared adhesion of H131 homozygous monocytes to R131 homozygous monocytes in a static adhesion assay
Binding of pan HLA I hIgG1 and hIgG2 to SAB and EC

Class I SAB all hIgG1v2

Normalized MFI

OD (hIgG bound to HAEC)

0.0 0.2 0.4 0.6 0.8 1.0 1.2

cell blank control hIgG HLA I hIgG1 100ng/mL HLA I hIgG2 100ng/mL

HLA I hIgG1

HLA I hIgG2
HLA I antibodies stimulate endothelial P-selectin

![Graph showing mean OD (P-selectin expression) for different treatments.]

** p<0.01, *** p<0.001 versus negative control

** P-selectin

** Ca²⁺

Weibel-Palade body
Is P-selectin required for monocyte adhesion in response to chimeric human HLA I antibodies?
Is P-selectin required for monocyte adhesion in response to chimeric human HLA I antibodies?

** Is P-selectin required for monocyte adhesion in response to chimeric human HLA I antibodies?**

- **HLA I hIgG1**
- **HLA I hIgG2**

*** p<0.001 versus untreated
† p<0.01, ‡ p<0.001 versus no inhibitor

- **Ca2+**
- **Weibel-Palade body**
- **PSGL-1**
- **P-selectin**
P-selectin is universally required for monocyte recruitment

* irrespective of HLA I antibody subclass (hIgG1 or hIgG2) or monocyte FcγRIIa allotype (H or R)
Monocytic Cell Lines Representing FcγRIIa Genotypes

R/R
U937

R/H
THP-1

H/H
Mono Mac 6

Monocytic cells were allotyped by flow cytometric method. Cells were stained with antibody recognizing total FcγRIIa, or the R131 allele of FcγRIIa (clone 3D3).

FcγRIIa

R131

H131

Monocytic cell lines representing FcγRIIa genotypes

R131

H131

[Graphs showing mean adherent cell counts for U937, THP-1, and Mono Mac 6 cells with and without immobilized IgG under different conditions (BSA, hlgG1, hlgG2) with significance indicated by asterisks (***)]
Are monocytes differentially recruited by HLA I antibodies depending on FcγRIIa alleles?

U937  R/R

![Graph showing mean adherent U937 per field](chart)

- Untreated
- HLA I hlgG1
- + anti-FcγRI
- + anti-FcγRIIa

** **** p<0.001 versus untreated
# p<0.05, ‡ p<0.001 versus no inhibitor
Are monocytes differentially recruited by HLA I antibodies depending on FcγRIIa alleles?

**U937**
- **R/R**
- **H/H**

**Mono Mac 6**
- **R/R**
- **H/H**
R131 monocytes bind hIgG1 predominantly through FcγRI

H131 monocytes bind hIgG1 through both FcγRI and FcγRII
Are monocytes differentially recruited by HLA I antibodies depending on FcγRIIa alleles?

**U937**

**R/R**

***p<0.001 versus untreated
ns p>0.05, ‡ p<0.001 versus no inhibitor
Are monocytes differentially recruited by HLA I antibodies depending on FcγRIIa alleles?

U937  R/R  Mono Mac 6  H/H
R131 monocytes bind hIgG2 through an FcγR-independent mechanism

H131 monocytes bind hIgG2 through FcγRII only
## Conclusions

<table>
<thead>
<tr>
<th>Monocyte</th>
<th>FcγRIIa Allele</th>
<th>FcγRIIa Affinity</th>
<th>Binding to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>hlgG1</td>
</tr>
<tr>
<td>MM6</td>
<td>H/H</td>
<td>High affinity</td>
<td>FcγRI +++</td>
</tr>
<tr>
<td>U937</td>
<td>R/R</td>
<td>Low affinity</td>
<td>FcγRI ++</td>
</tr>
</tbody>
</table>

### Diagrams

- **H131**
  - FcγRI
  - FcγRIIa
  - hlgG1

- **R131**
  - FcγRI
  - FcγRIIa
  - hlgG1

- **H131**
  - FcγRI
  - FcγRIIa
  - hlgG2
Implications

Understanding risk based on patient characteristics:

• FcγR polymorphisms in DSA+ transplant patients may influence outcome.
  – Recipients with FcγRIIa-H131 may experience greater infiltration of FcγR-bearing myeloid cells during AMR.
• IgG2 DSA may be clinically significant in H131 carrying recipients.

Strategies that antagonize the interaction of HLA IgG with FcγRs are likely to diminish macrophage, neutrophil and NK trafficking to the graft, reduce rejection and improve outcome.
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Luminex Single Antigen:
Monoclonal W6/32 hlgG1 chimer
Luminex Single Antigen

Class I SAB HLA I hlgG1

Class I SAB HLA I hlgG2
Pan-HLA I chimeric antibodies

The variable regions of mouse anti-HLA I (W6/32) Ab were cloned onto human constant regions to generate chimerized HLA I hIgG1 and hIgG2.