The role of antibody in chronic allograft dysfunction: animal models and human pathology

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Hammersmith Hospital
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Four conditions caused by antibodies

Hyperacute rejection
Acute humoral rejection
Chronic humoral rejection
Accommodation

All occur, as far as we know, by interaction of DSA with the graft endothelium

Colvin, JASN 18:1046, 2007
Chronic Antibody Mediated Rejection*

8.4% of renal transplant biopsies

1998-2008 MGH Farris et al
Chronic allograft arteriopathy
only in patients with "de novo" anti-donor antibodies
Missing link: antibody → pathology
Chronic Humoral Rejection: Identification of Antibody-Mediated Chronic Renal Allograft Rejection by C4d Deposits in Peritubular Capillaries

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“Chronic humoral rejection” = Transplant glomerulopathy or arteriopathy + C4d + DSA

38 pts → 61% C4d+ → 88% of C4d+ had DSA+
Classical complement pathway activation

Antibody + Antigen

C1

C4 → C4a + C4b

C4d

binds covalently to local site


C4d Peritubular Capillaries
C4d+ highly correlated with anti-donor HLA antibody

%C4d+ patients with anti-donor HLA Ab

95% Mauiyyedi JASN 13:779, 2002
88% Bohmig JASN 13:1091, 2002
92% Haas AJT 6:1829, 2006
92% Scornik AJT 7:1808, 2007
Transplant Glomerulopathy (TG)

C4d in PTC 32%

Antibody (DSA) 70% HLA Class II>I Ab (91% vs 61%)

PTC multilamination 91%

38% TG + DSA without C4d
Non C fix Ab?

26% TG without C4d or DSA
Non Ab cause (T cell, TMA/CNIT)
Residue of past Ab/C

Sis et al (Edmonton) AJT 7:1743, 2007
Criteria for chronic antibody-mediated rejection

1. Morphology
   Lamination of basement membrane glomeruli or PTC
   Arterial intimal fibrosis
   Interstitial fibrosis/tubular atrophy

2. Immunopathology
   C4d in PTC and/or glomeruli or Ig/C3 deposition

3. Serology
   Anti donor HLA or other endothelial antigens

2/3= Suspicious

Takamoto et al, Am J Transplant, 4:1033, 2004
Clinical Phenotype:
Chronic Humoral Rejection

8.4% of biopsies (1998-2008)
Mean time post-tx 8.4 ± 5.5 years
Mean Cr 4.1 ± 2.7 mg/dl
Proteinuria >1 gm/d 50%
Prior AHR 11%
One year graft survival 35% (0% at 5 yrs)

Farris et al (Boston), Mod Pathol, 2009
Pathological Features of CHR

• Transplant Glomerulopathy
  – GBM duplication
  – Capillary endothelial “dedifferentiation”- loss of fenestrations
  – Mononuclear cells in glomeruli
Cellular glomerulitis
Macrophages not T cells in glomeruli

CD8, GrzB (T)  

CD68 (Mac)
FcyRIII+ cells in capillaries

Farris et al USCAP 2009
TG Gene Expression

- TG (40) vs non-TG (54)*
  
- ↑Endothelial transcripts
  - VWF, DARC, MCAM, ICAM1
  
- ↑FcR
  - FcγRIIIA,B, FcγRIIA, FcγRIIB, FcεRIγ
  
- *matched for time post-tx

Sis et al (Edmonton) ASN, 2009
mRNA correlated with cells/mm²: r=0.61, p<0.01

T-bet+ cells in CHR

Ashton-Chess, Dugast, Colvin et al JASN 20:1113, 2009
FcγRIII+ T-bet+ cells in CHR

Brown = FcγRIII
Blue = T-bet

Farris et al USCAP 2009
IgG binding to FcγRIII* activates NK cells

**Diagram**

- IgG/Ag
- FcγRIII
- NK
  - ↑T-bet
  - ↑Cytoxicity
  - IFNγ
  - TNF-α, IL-8, MIP-1α, RANTES

*CDC16

Trotta et al JI 181:734, 2008; Roda et al, JI 177:120, 2006
Peritubular Capillaries

Mononuclear cells in PTC (capillaritis)

Multi-lamination of PTC basement membranes

Regele et al (Vienna)
JASN 13:2371, 2002
C4d+ associated with intracapillary mononuclear cells

Regele et al (Vienna) JASN 13:2371,2002
Peritubular Capillary Multilamination
PTC loss correlates with serum Cr

Ishii, Shimizu et al KI 67:321, 2005
Loss of PTC (CD34)

Non-tx

CHR
Range of C4d+ PTC density in CHR
1%-90%
Some CD34+ PTC are C4d-

Double stain C4d (brown-DAB) followed by CD34 (blue-AP)
Wide distribution of %C4d+ in CHR without obvious threshold.

N=15, mean 48% C4d+

Collins et al, Mod Pathol, 2009
C4d+ in late graft rejection assoc. with plasma cells
(52% vs 16%)*

*Coduval et al (Chicago) Transplant 79:228, 2005
Plasma cell in peritubular capillaries in CHR
Immunoglobulin Gene Transcripts (IGT) vs. time post-transplant

↑ intragraft synthesis of Ig is a feature of late graft dysfunction

Einecke AJT 8:1434, 2008
Local Production of DSA by cells in renal allografts

Thaunat et al, Transplant 85:1648, 2008
Serological Features of CHR
TG associated with Class II MHC Ab

MHC Specificity

I+II (81)

II only (54)

I only (58)

0 (330)

Gloor (Mayo) AJT 7:2124, 2007
Chronic Transplant Arteriopathy
Chronic Transplant Arteriopathy--Heart correlates with prior C4d+

80% with C4d $\rightarrow$ TA
27% without C4d $\rightarrow$ TA

p<0.001

Rodriguez et al (Cleveland)
AJT 5:2778, 2005
Time to do an experiment
Chronic Cardiac Transplant Arteriopathy in Mice: Relationship of Alloantibody, C4d Deposition and Neointimal Fibrosis

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B10.BR heart $\rightarrow$ B6/RAG1--/-

anti-H-2K\textsuperscript{\textkappa}

Control
Passive DSA to donor class I x 28d

B10.BR heart → B6.RAG1/-/ given anti-H-2Kk x28 d

Uehara et al AJT 27:5,2007
Antibody Effects on Endothelium

Colvin, Smith, Nat Immunol Rev 2005
Anti-class I DSA causes arteriopathy without complement activation in mouse heart allografts

Non-C’ fixing DSA

IgG1 → B6.RAG1^-/

C3 deficient recipient

IgG2a → B6.C3^-/- RAG1^-/

Pathogenesis of CHR

NK, MΦ FcR

IFNγ, TNFα...

Endothelial cell

C4d

Colvin, Smith, Nat Immunol Rev 2005
NK Cells in Ab mediated Tx Arteriopathy

Ly49g
Chronic Antibody Mediated Transplant Arteriopathy Dependent on NK Cells

P<0.005  P<0.001

Anti-H-2KkIgG2a (n=12)  Anti-H-2KkIgG2a + anti-NK1.1 (n=11)  No Rx (n=12)

Hirohashi et al, unpublished
Conclusions

Chronic humoral rejection does not require complement (vs Acute)

Effect requires NK cells
   Blocked by anti-NK 1.1

C4d stain does not detect this form of CHR

Hirohashi AJT 2010 and unpublished
Endothelial Gene Expression in kidneys with rejection and DSA

Transcripts include vWF, caveolin-1, E-selectin, CD31, CD34…

- Distinct pattern vs DSA− rejection
- Highest levels assoc with C4d+
- Significantly ↑ in C4d− DSA+ cases

Sis et al (Edmonton) AJT 9:2312,2009
DSA+ Endothelial Gene Expression = Poor prognosis

Explanation:
1) C4d stain insensitive
2) Complement independent mechanism

Sis et al. AJT 9:2312, 2009
“Normal” Protocol Biopsy with C4d+
C4d in Stable Renal Allografts
Protocol biopsies < 12 mo

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>C4d+</th>
</tr>
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<tbody>
<tr>
<td><strong>Routine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mengel (Hanover) AJT, 5: 1050, 2005</td>
<td>501</td>
<td>2-4%</td>
</tr>
<tr>
<td><strong>Pre-sensitized/HLA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haas (Hopkins) AJT, 6: 1829, 2006</td>
<td>103</td>
<td>17-26%</td>
</tr>
<tr>
<td><strong>ABO incompatible</strong></td>
<td></td>
<td></td>
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<tr>
<td>Grande (Mayo) AJT 4:101, 2004</td>
<td>16</td>
<td>25%</td>
</tr>
<tr>
<td>Haas AJT, 6: 1829, 2006</td>
<td>55</td>
<td>50-80%</td>
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</table>

Accommodation or early warning of rejection?
What exactly is accommodation and how do we recognize it?
Evidence for Peritubular Capillary Endothelial Accommodation *in vivo*

↑ bcl-xL in renal allografts from patients with donor specific HLA antibody

*Salama (Hammersmith) Am J Transplant 1:260, 2001*

↑ CD55 in stable vs unstable C4d+ cardiac grafts

*Gonzalez-Stawinski (Cleveland), JHLT 27:357, 2008*

↑ muc-1 gene expression/glom ABOi

*Park et al (Mayo) AJT 3:952, 2003*
Antibody Mediated Effects on Allografts

- Acute Rejection
- Chronic Rejection
- Stable

Accommodation strength

Rejection strength

Hyperacute Rejection
Time Course of CHR
Onset of TG

20% at 5 yr

Gloor (Mayo) AJT 7:2124, 2007
## Natural History of CHR

C4d+ predicts later glomerulopathy

<table>
<thead>
<tr>
<th>C4d</th>
<th>TG</th>
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<tbody>
<tr>
<td>Bx &lt;12 mo</td>
<td>Bx &gt; 12 mo</td>
</tr>
<tr>
<td>Neg</td>
<td>6%</td>
</tr>
<tr>
<td>Pos</td>
<td>46%</td>
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</table>

Regele et al (Vienna), JASN 13:2371,2002 (213 bx >12 m)
Antibody may fluctuate: Episodic C4d+/injury/repair

Cornell, Smith, Colvin, Ann Rev Pathol 2008
HLA antibody can disappear late in course

Recipient: A23,30,B41,57,DR8,13,DQ1,3
Donor: A23,36,B45,70,DR11,13,DQ1,6

DSA: B45
NDSA: B8, B44, B55, B72, B76, B82

DSA Titer (FI units)

Pathologic sequences observed in repeat biopsies

AHR → CHR (7 yr)
AHR → CHR → C4d- TG (18 mo)
AHR → CHR → C4d- TG (9 mo)

CHR → C4d- TG (6 mo)

C4d- TG → CHR (8 mo)

Collins et al, Mod Pathol, 2009
Time Sequences Observed

10% AHR → CHR

90% Insidious onset CHR
Animal Model of CHR

Cynomolgus monkey renal allgrafts
short term immunosuppression in conjunction with donor bone marrow

~50% long term graft acceptance
~50% CHR

Smith et al, AJT 2008
Similarity to human CHR

GBM duplication

PTC BM multilamination
Variants of mixed chimerism protocol
All off immunosuppression after day 30

48% developed anti-donor alloantibodies
29% C4d+ 22% TG+

143 Recipients
269 biopsies
5 nephrectomies
143 autopsies
Sequential Development of CHR

<table>
<thead>
<tr>
<th>No CHR</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>DAY 106</td>
<td>DAY 182</td>
<td>DAY 225</td>
<td>DAY 352</td>
</tr>
<tr>
<td></td>
<td>Cr = 1.2</td>
<td>Cr = 1.4</td>
<td>Cr = 1.8</td>
<td>Cr = 2.4</td>
</tr>
<tr>
<td></td>
<td>DAY 104</td>
<td>Ab Neg</td>
<td>DAY 182</td>
<td>Ab Pos</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DAY 225</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>DAY 352</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DAY 371</td>
<td></td>
</tr>
</tbody>
</table>

Days post-transplant:

- No CHR: 106, 182, 225, 352, 371

Smith et al (Boston) AJT 8:1662, 2008
## Postulated Stages of Humoral Rejection

<table>
<thead>
<tr>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
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**Accommodation**
- No C4d
- With C4d

**Rejection**
- Subclinical
- Clinical

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Sequential Development of CHR in Humans
Serial Renal Transplant Protocol Biopsies in a Patient
5 years
Cr 1.8 P/Cr 0.08

C4d
Outcome of CHR

Graft survival (post biopsy)

1 year 54%
2 year 24%
5 year 8%

N=50

Farris et al, submitted

Censored for death with a functioning graft
DeKAF Study
Outcome of patients with graft dysfunction + DSA + C4d

(bx for late graft dysfunction = 25%↑Cr, new proteinuria)

N=173

Graft Survival (%)

Months post-bx

Logrank = 23.20  p = 0.0000

~50% graft loss at 18 mo

Gaston et al Transplant 90:68,2010
Survival worse with combination of C4d+ and TG

Kieran et al (Seattle) JASN 20: 2260, 2009
Status
Chronic humoral rejection of kidneys

- Well defined pathologic criteria (Banff)
- Not uncommon cause of late graft dysfunction
- Outcome poor
- Earlier diagnosis needed
- Therapy yet to be defined
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