Antibody Mediated Rejection: Historical perspectives and animal models

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Banff, Alberta

Early Observations in Patients

1966 Hyperacute rejection

1967 Transplant glomerulopathy

1968 HLA antibodies post-transplantation

Humoral Antibodies in Renal Allotransplantation in Man

Chronic allograft arteriopathy
only in patients with de novo anti-donor antibodies

Dark Ages for Antibody
1970-1990
No connection between antibodies and pathology
Antibodies regarded as epiphenomenon
T cells reigned

Antibodies affect outcome of acute rejection and pathologic pattern

1990

1992

1996
Sunrises can be foggy in Canada

Vascular deposition of complement-split products in kidney allografts with cell-mediated rejection

C. E. FEUCHT†, 1, E. FELBER, M. J. GOELLE, G. HILLERBRAND, H. NATTERMANN, C. BROCKMAYER, E. HILD, G. RITTSCHILLER, W. LANZ, 1, 2, E. ALBERT. Departments of Pathology, University of Munich, Munich, Germany, and Medizinische Klinik II, Freie Universität Berlin, Berlin, Germany.

SUMMARY

Complement activation in transplant rejection is monitored by immunoperoxidase detection using antibody to complement component C4d. Deposition of complement fragment C4d in peritubular capillaries, indicating activation of classical pathway, could be detected in the majority of transplant kidneys with cell-mediated rejection. Alloantibody deposition of complement-split products was observed in 21 early biopsies from patients with high immunological risk. Despite negative results in the crossmatch before transplantation and positive immunoglobulin in transplanted biopsies, alloantibodies directed against mismatched self antigens should be considered as a possible cause of classical complement activation.

C4d Deposition detected in PTC in Rejection

"On the basis of covalent binding and amplification, it appears that staining of complement fragment C4d seems to be a practical tool for the demonstration of in situ humoral immune reactions that are not easily detectable otherwise."

Helmut Feucht et al, 1991

<table>
<thead>
<tr>
<th>Table 2. Staining pattern and intensity of deposited complement fragment C4d within transplant biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Localization of complement C4d</td>
</tr>
<tr>
<td>a</td>
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<tr>
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</tr>
<tr>
<td>Acute cell-mediated rejection</td>
</tr>
<tr>
<td>20</td>
</tr>
<tr>
<td>18</td>
</tr>
<tr>
<td>(early rejection)</td>
</tr>
<tr>
<td>15</td>
</tr>
<tr>
<td>7</td>
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<tr>
<td>4</td>
</tr>
<tr>
<td>Chronic rejection</td>
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<tr>
<td>6</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Cyclosporin therapy</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>Ischemic damage</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>Recurrent glomerulonephritis</td>
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<tr>
<td>1</td>
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</tbody>
</table>

Range of staining intensity: -, absent; +, moderate; ++, strong; ++++, very strong; PTC, peritubular capillaries; Art, arteriole; Glom, glomeruli; Tab, tubular basement membranes.
* Fixed intensity, >80%.

Connect the dots

Graft Pathology

C4d
PTC

DSA

Connect the dots

C4d
PTC

DSA

DSA = Donor Specific Antibody (HLA)
Complement Activation in Acute Humoral Renal Allograft Rejection: Diagnostic Significance of C4d Deposits in Peritubular Capillaries

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Abbreviations: C4d, C4d, peritubular capillaries

C4d correlated with C4d+ and polys in caps

Chronic humoral rejection

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

38 pts → 61% C4d+ → 88% of C4d+ had DSA+

Other key observations

C4d Deposition is transient (days)
Indicates current antibody activity

Nolte J, Urena M, Guzman F, Thai G, and Mihatsch MJ.

Shintani K, Muniz K, Lee CY, Fan-Talbot K, Wasowska SA, Pescovitz MD, and Baldini WM,

ABOi grafts can have C4d without pathology

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ABOi grafts can have C4d without pathology


Sunrise Banff 2001

New Banff Categories

2001 Acute Ab Mediated Rejection

2005 Chronic Ab Mediated Rejection

2007 C4d deposition without active rejection

Technical Advances

Polyclonal anti-C4d for FFPE tissue
Useful for evaluation of glomeruli

Solid Phase Assays for HLA DSA
Donor cells not needed, sensitive, specific

ELISA

Luminex

C4d has provided a missing link: antibody → pathology
Terasaki et al AJT 7:408, 2007

Complement fixing alloantibodies detected on Ag coated beads (FlowPRA, Luminex)

AntiC4d

Incubate in fresh normal serum

More sensitive than cellular methods ~4x more presens detected
Wahrman (Vienna) AJT 7:1033, 2007

C4d fixation by pretransplant sera on Class I beads correlates with outcome

Strong correlation with C4d in bx
Wahrman (Vienna) AJT 7:1033, 2007
Intragraft events

**Immunoglobulin Gene Transcripts (IGT)**

**vs. time post-transplant**

<table>
<thead>
<tr>
<th>IGT Score (log₂)</th>
<th>Intragraft synthesis of Ig is a feature of late graft dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time post Tx (months)</td>
<td>r = 0.613**</td>
</tr>
</tbody>
</table>

Einbecke (Edmonton) AJT 8:1434, 2008

Local Production of DSA by cells in renal allografts

**Thaunat et al (Lyon), Transplant 85:1648, 2008**

Transplant Glomerulopathy (TG)

<table>
<thead>
<tr>
<th>Antibody (DSA)</th>
<th>70% HLA Class II&gt;I Ab (91% vs 61%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4d in PTC</td>
<td>32%</td>
</tr>
<tr>
<td>PTC multilamination</td>
<td>91%</td>
</tr>
</tbody>
</table>

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

Perplexing Heterogeneity

Subsets of CHR revealed by cluster analysis
Chronic disease evolves over time
Sequential events in CHR

C4d deposition predicts later transplant glomerulopathy

<table>
<thead>
<tr>
<th>C4d</th>
<th>TG</th>
</tr>
</thead>
<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>
</tr>
</tbody>
</table>

Regele et al (Vienna), JASN 13:2371-2002 (213 bx >12 m)

↑ risk of TG after episode of AHR

Prevalence of TG in 1 yr protocol bx

- Conventional 198 8%
- Presens HLAi 37 22% p<.015
- ABOi 24 13%

- Prior AHR risk factor for TG (OR 17.5, multivariate)
- Also for arteriopathy and interstitial fibrosis
- Presensitized patients and possibly ABOi increased risk

Gloor et al (Mayo) AJT 6:1841, 2006

Pathologic sequences observed in repeat biopsies

AHR → CHR (7 y')
AHR → CHR → C4d- TG (18 mo)
AHR → C4d- TG (9 mo)
C4d- TG → CHR (8 mo)

Collins et al, Mod Pathol, 2009

Postulated Stages of Humoral Rejection

<table>
<thead>
<tr>
<th>Stage</th>
<th>Transplant</th>
<th>Accommodation</th>
<th>Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>No C4d</td>
<td>Clinical</td>
<td>Clinical</td>
</tr>
<tr>
<td>II</td>
<td>With C4d</td>
<td>Subclinical</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>IV</td>
<td></td>
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</tbody>
</table>

Colvin and Smith, Nature Rev Immunol 5:887, 2005

Other Organs
C4d Publications by Year

Accepted Organ Specific Criteria for Antibody Mediated Graft Rejection

Cardiac Allograft Vasculopathy (CAV)

Even the liver can have AMR

C4d Rosetta Stone

Liver is less susceptible to CHR than Kidney
Endothelium: Target and Defender

Anti-HLA antibodies trigger endothelial responses

Induction of High Affinity Fibroblast Growth Factor Receptor Expression and Proliferation in Human Endothelial Cells by Anti-HLA Antibodies: A Possible Mechanism for Transplant Atherosclerosis

Paul E. Harris, Hong Bin, and Elaine F. Reed

The major limitation to long-term survival of organ allografts is chronic rejection, which is mediated at least in part by the growth of the transplanted organ. There is significant evidence that transplanted cryopreserved endothelial cells (ECs) and transplant rejection with calcineurin inhibitors. This, in turn, leads to chronic rejection of EC, and new vessel wall is also accompanied by cytokine and chemokine. These data suggest that the cellular rejection observed in transplant rejection is the result of the proliferative effects of anti-HLA Ab. The Journal of Immunology, 1997; 154:591-596.

Phosphorylated signaling proteins
In vivo markers of endothelial activation

Phosphorylated 86-Ribosomal Protein: A Novel Biomarker of Antibody-Mediated Rejection in Heart Allografts


What role do cells play?

Macrophages enhance cytokine production by endothelial cells triggered by anti-MHC I Ab

Endothelial cells

DSA alone

DSA + Macs

MCP-1, KC

MCP-1, KC, IL-6, Rantes, TIMP-1

FcRγIII KO macrophages less active, as were F(ab)_2 DSA
Endothelial cells resist effects of antibody/complement

- ↑ bcl-xL in renal allografts from patients with donor specific HLA antibody
  Salama (Hammersmith) Am J Transplant 1:260, 2001

- ↑ muc-1 gene expression/glom ABOi
  Park et al (Mayo) AJT 3:952, 2003

- ↑ CD55 (DAF) in stable vs unstable cardiac grafts
  Gonzalez-Stawinski et al (Cleveland) JHLT 27:357, 2008

Capillary DAF+ identifies C4d+ biopsies with no graft dysfunction

DAF = decay accelerating factor, CD55

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

†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
- Low sensitivity of C4d stain vs non-C’ fixing effects of DSA

Sis et al (Edmonton) AJT in press

Experimental Studies

Animal Models of AHR/CHR

Xenograft rejection
  Pig to monkey/baboon
  Rat heart to α-gal KO mouse

AHR
  Rat (C6) (Baldwin)
  Mouse heart (Wasowska)

CHR
  Mouse heart allograft
  Mouse with human artery
  Monkey renal allograft

AHR depends on complement fixation

Mouse heart allografts
B10.A hearts → B6 Ig KO

Complement fixing IgG2b DSA triggered immediate rejection at 10 days, but non-C’ fix IgG1 did not.

Chronic Arteriopathy can be caused by DSA

Non-complement fixing antibody (IgG1) can initiate chronic allograft arteriopathy

Anti-H-2Kk mAb B10BR hearts in B6

Alloantibody can cause one feature of chronic humoral rejection without complement (NK/FcR?)

May be relevant to cases with DSA and chronic rejection without C4d
### Human arterial segment transplanted into immunodeficient mouse (SCID/Beige)

Lorber, Tellides, Pober (Yale)
*Transplant 67:897, 1999*

Thomsen (Toulouse)-mesenteric
*JHLT 25:675, 2006*

Galvini (Toulouse)
in press AJT
Anti-HLA class I → arteriopathy in 6 wk

### Non-human Primate Renal Allografts

Knechtle
Larsen, Kirk
Thomas
Jonker
Wieczorek, Mihatsch, Nickeleit

Cosimi, Kawai

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**Four Stages and Lack of Stable Accommodation in Chronic Alloantibody-Mediated Renal Allograft Rejection in Cynomolgus Monkeys**

Departments of Pathology and Surgery, Massachusetts General Hospital, Boston, MA, and Harvard Medical School, Boston, MA.

*Variants of mixed chimerism protocol*
All off immunosuppression after day 30
48% developed anti-donor alloantibodies
29% C4d+ 22% TG+

143 Recipients
269 biopsies (protocol/indication)
5 nephrectomies
143 autopsies

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**CHR in Cynomolgus Renal Allograft C4d+, DSA+**

Smith et al, AJT 6:1790, 2006

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**Similarity to human CHR**

- GBM duplication
- PTC BM multilamination

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**Kaplan - Meier Survival Curves**

Renal allograft loss due to rejection- N=143
Non-human primates off immunosuppression

Smith et al, (Boston) AJT 8:1662, 2008
Sequential Development of CHR-Monkeys

<table>
<thead>
<tr>
<th>No CHR</th>
<th>Stage I</th>
<th>Stage II</th>
<th>Stage III</th>
<th>Stage IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days post-transplant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>106</td>
<td>182</td>
<td>225</td>
<td>352</td>
<td>371</td>
</tr>
</tbody>
</table>

Smith et al (Boston) AJT 8:1662, 2008

Future Questions

**Basic**
- Regulation of B cell function
- Endothelial mechanisms
- Mechanism of accommodation
- Role of complement and FcR/cells

**Clinical**
- Natural history
- Monitoring Ab, intervention
- New therapies (Plasma cell, B cell, C')

Transplant Research MGH

**Calvin Lab/Pathology**
- R. Neal Smith
- Sandra Alessandri
- Brad Ferris
- Clara Taheri
- Tricia Della Pelle
- Nicole Broussacade
- Bernard Collin
- Catherine Applebaum

**Multicenter Trials**
- CTOT
- ITN
- Genomics of Tx
- CTOT-C

**Harvard Collaborators**
- Ab Saumyth
- Terry Strom

**Clinical**
- Susan Sashman
- Donna Fitzpatrick

**Monkey**
- Ben Cousins
- Jim Markman
- Joren Medsen
- New Tinkoff/Runner
- Neilh God
- Eliot Taller
- Lora Williams
- Tatsuo Kawai
- Frank Gandelman
- Jay Feinman
- Megan Sylve
- Paul Pfeffer

**CTOT-C**
- Paul Russell
- Cuffy Charles
- Joren Medsen
- Tommy Nathans
- Max Miyagawa

High Noon for AMR

Late Graft Failure

Abominable Antibody

(this guy is bigger)