Antibody Mediated Renal Allograft Rejection

V. Nickeleit, MD
The University of North Carolina at Chapel Hill
Nephropathology Laboratory
Chapel Hill, N.C. 27599 (USA)
Email: volker_nickeleit@med.unc.edu
Time of / for confusion........
What do you understand under the term: antibody mediated renal allograft rejection?
“The primary biopsy features of antibody mediated rejection are the detection of C4d combined with evidence of renal allograft injury”.... (and the detection of circulating donor specific antibodies).

This definition is problematic since it suggests that circulating antibodies & C4d positivity mark THE (humoral) cause for graft injury.

Concomitant cellular rejection or antibody mediated injury superimposed on other diseases such as calcineurin-inhibitor toxicity, pre-existing donor disease etc. remain largely unclassified.....
What do you understand under the term: antibody mediated renal allograft rejection?
I prefer the following definition:

Alloimmune responses with a C4d positive antibody mediated component that ranges from dominant -to- co-dominant -to- minor.
Case 1

The patient is a 31 year-old African American male with end-stage renal disease due to “FSGS”. He underwent his 2nd cadaveric renal transplantation in February of 2003.

Renal function after transplantation improved rapidly under ATG induction (baseline serum creatinine reading of 3.4 mg/dl). Subsequent rise of S-Cr to 6mg/dl and evidence of proteinuria (3+). A diagnostic graft biopsy was performed on day 18 post transplantation.
Case 1 – Image 1

Where is the action?
What do the tubules show?
What do you diagnose in image #1?
Case 1

T = Tubulus
   (arrow heads at basement membrane)

G = Glomerulus
Case 1 – Image 1

Where is the action?
Answer: in the tubulo-interstitial compartment with edema and predominately mononuclear inflammatory cell infiltrates.

What do the tubules show?
Answer: tubulitis

What do you diagnose in image # 1?
Answer: tubulo-interstitial cellular rejection.
Case 1 – Image 2

What is the anatomic structure in the middle of this image?

Is this structure normal or abnormal?

Does the finding in image # 2 change your diagnosis?
Case 1 – Image 2

What is the anatomic structure in the middle of this image?

**Answer:** a (large) interlobular type artery.

Is this structure normal or abnormal?

**Answer:** abnormal. It shows inflammation of the intimal layer (transplant endarteritis) superimposed on mild donor disease (pre-existing arteriosclerosis).

Does the finding in image # 2 change your diagnosis?

**Answer:** yes. Rejection episodes with transplant endarteritis are more severe than those with ‘only’ tubulo-interstitial cellular rejection.
Immunofluorescence microscopy: MHC-class II (HLA-DR) incubation
Case 1 – Image 4

Immunofluorescence microscopy:
C4d incubation
Case 1 – Images 3 and 4

Illustrated here are immunofluorescence incubations to detect the MHC-class II (HLA-DR) expression in tubular epithelial cells and the accumulation of C4d along peritubular capillaries.

- Do images #3 and #4 show normal or pathological findings?

- What is your final diagnosis and how do you treat the patient?
Immunofluorescence microscopy: MHC-class II (HLA-DR) incubation
Immunofluorescence microscopy: C4d incubation
Case 1 – Images 3 and 4

Do images #3 and #4 show normal or pathological findings?

Answer: abnormal. As expected in cellular rejection episodes (transplant endarteritis/tubulo-interstitial cellular rejection) tubular epithelial cells express MHC-class II (HLA-DR).

Unexpected in this case is the detection of the complement degradation product C4d along peritubular capillaries marking a concurrent antibody mediated rejection component.
Case 1:

Subsequent studies showed high titers of circulating anti-donor class I antibodies (anti A2).
Case 1 – Images 3 and 4

What is your final diagnosis and how do you treat the patient?
Answer: 1) cellular rejection with transplant endarteritis, C4d positive (mixed cellular and antibody mediated rejection)

(Banff category 4, type II rejection and category 2 rejection).

2) Anti-lymphocytic sera plus IVIG, plasmapheresis, immunoabsorption …
Case 1:

Should this case be simply labeled “antibody mediated rejection” ….?

NO!!!
Remember “my” definition:

Alloimmune responses with a C4d positive antibody mediated component that ranges from dominant -to- co-dominant -to- minor.

Case #1 would represent a “co-dominant” antibody response closely linked to severe cellular rejection with endarteritis.
Case 2

The patient is a 43 year-old African American male with end-stage renal disease of “undetermined etiology”. He underwent cadaveric renal transplantation in October of 2003.

Renal function after transplantation improved rapidly. Subsequent rise of S-Cr to 6.1 mg/dl, no evidence of proteinuria. A diagnostic graft biopsy was performed on day 7 post transplantation.
Case 2 – Images # 1 and # 2

T = Tubulus
G = Glomerulus
V = Artery
I = Interstitium

- Is the interstitial inflammatory response purely lymphocytic (compare with case 1) ?
- Are the tubules and the artery normal ?
- What do the arrows mark in the glomerulus ?
Is the interstitial inflammatory response purely lymphocytic (compare with case 1) ?

**Answer:** no. The inflammatory infiltrate is relatively sparse and consists of lymphocytes and “many” polymorphonuclear leukocytes.

Are the tubules and the artery normal ?

**Answer:** yes. There is no tubulitis, only mild acute tubular injury.

What do the arrows mark in the glomerulus ?

**Answer:** small microthrombi. Note that the arteries are uninvolved.
Immunofluorescence microscopy: MHC-class II (HLA-DR) incubation
Immunofluorescence microscopy:
C4d incubation

Case 2 – Image 4
Case 2 – Images 3 and 4

Illustrated here are immunofluorescence incubations to detect the MHC-class II (HLA-DR) expression in tubular epithelial cells and the accumulation of C4d along peritubular capillaries.

- Do images #3 and #4 show normal or pathological findings (compare to case 1)?

- What is your final diagnosis and how do you treat the patient?
Immunofluorescence microscopy: MHC-class II (HLA-DR) incubation

Case 2 – Image 3
Immunofluorescence microscopy:
C4d incubation
Case 2 – Images 3 and 4

Do images #3 and #4 show normal or pathological findings (compare to case 1) ?

**Answer:** In contrast to case 1, tubules do not express MHC-class II (HLA-DR). This is expected in a case lacking tubulitis and a cellular rejection component.

The C4d staining illustrated in image 4 is abnormal; it shows diffuse capillary C4d accumulations along peritubular capillaries.
Case 2:
Subsequent studies showed high titers of circulating anti-donor class II antibodies.
Case 2 – Images 3 and 4

What is your final diagnosis and how do you treat the patient?
Case 2 – Images 3 and 4

**Answer:**

1) Capillary transplant rejection, C4d positive (pure antibody mediated rejection) *(Banff category 2 rejection).*

2) Strategies to eliminate the circulating antibodies, i.e. plamapheresis, IVIG, immunoabsorption etc....
Remember “my” definition:

Alloimmune responses with a C4d positive antibody mediated component that ranges from dominant -to- co-dominant -to- minor.

Case #2 would represent a “dominant” antibody response.
Antibody Mediated Rejection & C4d Concepts and Confusion

- Background
- C4d and circulating Antibodies
- C4d and Histology
- C4d – Terminology and Classification of Rejection
- Questions & Quiz
Antibody Mediated Rejection & C4d Concepts and Confusion

- **Background**
- C4d and circulating Antibodies
- C4d and Histology
- C4d – Terminology and Classification of Rejection
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Fascination

C4d: finally an immunohistochemical marker for an antibody mediated allo-response!
C4d is Not “New”!

C4d pioneers:
The group of H.E. Feucht from Munich

Kid Int (36): 1069-1077, 1989
Immunology (80): 162-167, 1993
Kid Int (50): 1464-1475, 1996
Classical Pathway

Antigen-antibody Complexes

C1qrs → C1q → C4 → C4a → C4b → C4c → C4d

Microorganisms Mannan-binding-lectin Pathway

C3 → C3a → MAC
C3b → C3c → C3d

Alternative Pathway
C4d

positive

negative
C4d significant independent marker for poor graft survival

Feucht et al. Kid Int (43): 1333-1338, 1993
Herzenberg, Magil et al. JASN (13): 234-241, 2002
Böhmic, Regele et al. JASN (13): 1091-1099, 2002
The graph shows the percentage of graft survival over time for different groups:

- **C4d** group with 42 participants: 90% at 12 months.
- **Total** group with 93 participants: 72% at 12 months.
- **C4d (+)** group with 8 participants: 63% at 12 months.
- **C4d +** group with 43 participants: 57% at 12 months.

The x-axis represents time in months, ranging from 0 to 12, and the y-axis represents the percentage of graft survival, ranging from 0% to 100%.

Feucht et al. 1993
C4d

- Specific for (renal) transplants
- Highly exceptional in native kidneys (Anti-GBM Disease, Lupus Nephritis)
- Easily detected by immunohistochemistry
- Currently, no evidence of mannann-lectin activation*

Interpreted as a footprint of an antibody mediated allo-response

Antibody Mediated Rejection & C4d Concepts and Confusion

- Background
- **C4d and circulating Antibodies**
- C4d and Histology
- C4d – Terminology and Classification of Rejection
- Questions & Quiz
Flow-cytometric crossmatch (FCXM)

C4d positive (n=24)  C4d negative (n=89)
FCXM neg. 3 (12.5%)  42 (47.2%)
FCXM pos. 21 (87.5%)  47 (52.8%)

\(X^2\) P<0.01

antibody specificity against HLA-class I and / or class II

*JASN* (13): 1091-1099, 2002
Circulating Donor Specific Antibodies

C4d Positivity

- DSA pos C4d neg
- DSA pos C4d pos
- DSA neg C4d pos
C4d and unusual circulating antibodies
C4d positivity, rejection and antibodies targeting angiotensin II type 1 receptor

Case provided by Prof. K. Amann, Erlangen
Antibody Mediated Rejection & C4d Concepts and Confusion

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(“Diagnostic”) C4d is deposited mainly along the intact endothelial cell layer of peritubular capillaries in the renal cortex and / or the renal medulla
C4d and Histology

Detection:

1) Immunofluorescence (IF) microscopy on frozen tissue
   Advantage:  - strong staining
                - no background
                - internal staining control
                  (mesangial regions normally positive)
   Disadvantage: - frozen tissue required

2) Immunohistochemistry (IHC) on formalin fixed tissue
   Advantage:  - tissue availability
                - superior morphology
   Disadvantage: - weaker staining
                 - no internal staining control
                   (mesangial regions negative)
                 - special antigen retrieval required
                   (e.g. pressure, steam)
Comparative Analysis of Two different C4d Detection Methods:

1) Immunofluorescence Microscopy on frozen tissue versus

2) Immunohistochemistry on Formalin fixed Tissue

?.....Are the (diagnostic) results identical.....?
<table>
<thead>
<tr>
<th>C4d incubations</th>
<th>Immunofluorescence: frozen tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse</td>
<td>Diffuse</td>
</tr>
<tr>
<td>Diffuse</td>
<td>18</td>
</tr>
<tr>
<td>Focal</td>
<td>6</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
<tr>
<td>Total N</td>
<td>26</td>
</tr>
</tbody>
</table>

IHC negative cases: 60% positive by IF

Kappa value: 0.32

Comparative Analysis of Two different C4d Detection Methods:

?.....Are the (diagnostic) results identical?....?

No! Frozen tissue and immunofluorescence microscopy are more sensitive tools to detect C4d along capillaries.
In renal specimens C4d deposits can be found in the setting of many different histologic changes; they are often seen with acute cellular rejection.
<table>
<thead>
<tr>
<th>Histology</th>
<th>C4d positive / total</th>
<th>X² p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Tx glomerulitis</td>
<td>57% (28/49)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>• Tubular MHC-Class II expression</td>
<td>41% (102/246)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>• Interstitial rejection</td>
<td>43% (56/131)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>• Tx endarteritis</td>
<td>45% (33/74)</td>
<td>0.004</td>
</tr>
<tr>
<td>• Inactive intimal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sclerosis</td>
<td>33% (16/48)</td>
<td>ns</td>
</tr>
<tr>
<td>• Tx glomerulopathy</td>
<td>53% (9/17)</td>
<td>0.07</td>
</tr>
<tr>
<td>• Cyclosporine tox.</td>
<td>28% (40/145)</td>
<td>ns</td>
</tr>
<tr>
<td>• BK-Virus nephropathy</td>
<td>0% (0/9)</td>
<td>NA</td>
</tr>
<tr>
<td>• Fibrinoid vascular necrosis (v3)</td>
<td>50% (5/10)</td>
<td>NA</td>
</tr>
<tr>
<td>• Acute tubular necrosis</td>
<td>27% (3/11)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*JASN (13): 242-251, 2002*
C4d and Banff types I and II rejection

• Category 4, type 1 rejection (tubulo-interstitial)  
  24% to 43% of cases C4d positive

• Category 4, type 2 rejection (tx endarteritis)  
  approx. 45% of cases C4d positive

Cellular Response Antibody Response

Mixed Cellular and Antibody Rejection

Pure Cellular Rejection  Mixed Cellular and Antibody Rejection  Pure Antibody Rejection
C4d positivity

- Acute cellular rejection
- Chronic rejection
- Pure humoral rejection
- Other changes
- Normal histology
Does “my” definition of antibody mediated rejection make more sense now.....?

Alloimmune responses with a C4d positive antibody mediated component that ranges from dominant -to- co-dominant -to- minor.
“Pure” antibody mediated rejection

(Capillary Transplant Rejection, C4d positive)

These are rare rejection episodes (see case #2) caused by a DOMINANT antibody response lacking a cellular component. Such rejections account for approximately 5%-10% of all acute rejection episodes and primarily target capillaries.
Pure Antibody mediated Rejection
Capillary Transplant Vasculopathy

*Pure Antibody mediated Rejection*

- Strong diffuse C4d staining along capillaries
  - Capillaries (glomeruli / peritubular capillaries)
    - Dilated
    - Filled with polymorphonuclear leukocytes and / or monocytes
    - Focal fibrin thrombi
    - Interstitial edema and focal hemorrhage
- Acute tubular injury
- No evidence of cellular rejection
- No tubular MHC-class II expression
- No transplant endarteritis / no large thrombi
Antibody Mediated Rejection & C4d Concepts and Confusion

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How to classify C4d positive biopsies (nomenclature)?
C4d and Terminology

How to classify C4d positivity (nomenclature):

a) as a subclassifier to “standard” histological terms?

b) as “acute humoral” or “chronic humoral” rejection, i.e. the updated Banff ’97 scheme (2005 revision)?
Antibody-mediated rejection (Banff category 2)

1) Serological evidence of anti-donor antibodies

2) Morphology and immunohistochemistry
   A) Acute antibody mediated rejection
      I. ATN like: minimal inflammation, C4d positive
      II. Capillary: Tx glomerulitis, inflammatory cells in peritubular capillaries, thrombi, C4d positive
      III. Arterial: fibrinoid vascular necrosis (v3), C4d positive
   
   B) Chronic antibody mediated rejection
      GBM duplication and/or peritubular capillary basement membrane multilayering and/or fibrosis with tubular atrophy and/or intimal sclerosis, C4d positive
The Banff categories are broad and largely non-specific for antibody mediated graft injury.

The Banff categories do not necessarily mark all relevant pathways that resulted in graft damage.

The (only) unifying factor reflecting the contribution of antibodies in graft injury is the detection of C4d!

Thus:......
At this juncture, is it really helpful to create imprecise “new” Banff categories considering our currently limited knowledge of “C4d-positivity” and “antibody mediated” graft injury…?
C4d and Terminology

How to classify C4d positivity (nomenclature):

a) as a subclassifier to “standard” histological terms !
   (See diagnoses for cases #1 and 2)

b) as “acute humoral” or “chronic humoral” rejection,
   i.e. the updated Banff ’97 scheme (2005 revision) ?
Is “my” definition of antibody mediated rejection convincing now…..?

Alloimmune responses with a C4d positive antibody mediated component that ranges from dominant -to- co-dominant -to- minor.
C4d positivity

- Acute cellular rejection
- Chronic rejection
- Pure humoral rejection
- Other changes
- Normal histology
Antibody Mediated Rejection & C4d Concepts and Confusion

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Quiz...........
Transplant Glomerulopathy
Transplant Glomerulopathy generally marks “antibody mediated rejection”.

False - or - correct ?
Answer: false.

40%-50% of biopsies with transplant glomerulopathy are C4d negative. Transplant glomerulopathy can be induced by cellular and/or antibody mediated rejection. Identical glomerular changes are seen in thrombotic microangiopathies or secondary to calcineurin inhibitor toxicity.
Illustrated here is a case of delayed graft function with marked acute tubular injury.

Is this histologic change sufficient for rendering the diagnosis of ‘antibody mediated rejection (Banff category II, type 1 “ATN-like”) ?
Answer: no!

Only a minority of cases with “ATN” are caused by antibody mediated rejection! In order to characterize this case as “Banff category II rejection”, positive C4d staining and ideally also the detection of circulating donor specific antibodies are required.
Alloantibody response

- Cellular rejection
- Sclerosing rejection
- Pure T cell-poor, antibody-mediated rejection
- Other changes, e.g., calcineurin inhibitor toxicity, etc.
- Normal histology
• Classify rejection according to morphologic changes.

• Use C4d as a diagnostic qualifier.
Classification schemes come and go........even “Banff”.....

Morphologic patterns of diseases remain the same.....