Pathology of the late post-transplant kidney and the role of non-T cells

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Outline

• Causes of chronic injury in renal allografts
• Review of “non-T” cells in kidney transplant rejection (*late* rejection)
• Late post-transplant protocol biopsies, insights into late graft dysfunction and loss
Potential causes of late/chronic graft injury

- Donor-specific antibody/AMR
- Chronic inflammation (i-IFTA, IFTA+i)
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Potential causes of late/chronic graft injury

- Donor-specific antibody/AMR
- Chronic inflammation (i-IFTA, IFTA+i)
- Glomerular disease (including TG, recurrent dis)
- Progressive IFTA?
- Other?

Causes of graft loss in 153 conventional kidney transplants

Indication biopsy diagnosis by time post-transplant

Subclinical graft inflammation

• “IFTA+i” present in ~15% of 1 year protocol biopsies

• Increased staining for CD3, CD68 in IFTA+i

![Graphs showing increased immunostaining for T cells (CD3) and macrophages/DCs (CD68) in 1 year protocol biopsies with IF+i by Banff '97 criteria.]

Subclinical graft inflammation

Association with later development of IFTA

• ? Form of cell-mediated rejection

RT–PCR arrays: IFTA+i associated with increased activity of innate immune pathways including IFN-γ and Toll-like receptor responses, and T cell immunity

Whole-genome microarrays: cytotoxic T lymphocytes, IFN-γ response, B cells, and acute rejection signatures in IFTA+i compared with normal and IF-alone groups

“Non-T cells” in kidney transplants

- B cells and plasma cells
- NK cells
- Monocytes/macrophages
- Mast cells
B cells

Several modes of graft injury:

Antibody-mediated rejection:

• B cells and plasma cells produce donor-specific antibody (DSA)
• B cell differentiation to plasma cells

Li XC. The significance of non-T-cell pathways in graft rejection: implications for transplant tolerance. Transplantation. 2010 Nov 27
B cells

Several modes of graft injury:

Antibody-mediated rejection:

• B cells and plasma cells produce donor-specific antibody (DSA)
• B cell differentiation to plasma cells

Serve as antigen-presenting cells (APCs) to T cells

• Generation of alloreactive T cells, memory T cells
• Secretion of cytokines (pro- and anti-inflammatory)
• May also be involved in tolerance

Li XC. The significance of non-T-cell pathways in graft rejection: implications for transplant tolerance. Transplantation. 2010 Nov 27
Adams AB, Newell KA. B cells in clinical transplantation tolerance. Semin Immunol. 2012 Apr
B cells infiltrating the allograft

• Dense clusters of B cells in renal biopsies associated with severe graft rejection


• Other studies showed conflicting results
B cells infiltrating the allograft

Mouse kidney transplant model:

• Mature B cells recruited to the kidney transplant, form tertiary lymphoid tissue; progressive IFTA

• B cell depletion attenuates these changes

• Ex vivo culture of isolated intra-allograft B cells:
  • Supernatant showed significant levels of T cell chemokines, monocyte cytokines, TGF-β, other profibrotic cytokines

→ Mechanism of B cell damage related to cytokine production

Plasma cell-rich rejection
Plasma cell-rich rejection

Associated with:

- Resistance to rejection treatment, adverse outcome
- Medication non-adherence
- DSA, C4d deposition, capillaritis, TG
- IFTA, interstitial inflammation

Mixed AMR/TCMR


Plasma cell-rich acute rejection with alemtuzumab induction

- **Biopsy**: Plasma cell-rich acute rejection (~10% are C4d+)
- Alemtuzumab-induced lymphocyte depletion leads to dominance of naïve B cells
- Unique phenotype may be due to a different B cell repertoire that develops
  - Potentially has a different response to conventional anti-rejection therapy, other types of plasma cell-rich rejection

P Zhang, H Amer, CA Schinstock, MP Alexander, FG Cosio, MD Stegall, LD Cornell, Acute Rejection After Alemtuzumab Induction in Kidney Transplant Recipients. ATC abstract 2017
Monocytes/macrophages and rejection

- A component of inflammatory infiltrate in rejection (AMR, TCMR)
- Produce pro-inflammatory cytokines (IL-1, IL-6, TNF-α)
- Immune regulation
- Antigen presentation
- Tissue remodeling
Inflammation in +XM transplants at 5 years

- Persistent inflammation (capillaritis) and TG in +XM transplants

Chronic humoral rejection may occur in the absence of complement deposition in the tissue injury may be due to persistent inflammatory infiltrate.

<table>
<thead>
<tr>
<th></th>
<th>Conventional</th>
<th>ABOi</th>
<th>+XM</th>
<th>Conv/ABOi</th>
<th>Conv/+XM</th>
<th>+XM/ABOi</th>
</tr>
</thead>
<tbody>
<tr>
<td>cg</td>
<td>7.6%</td>
<td>12%</td>
<td>59.5%</td>
<td>0.457</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ptc</td>
<td>8.9%</td>
<td>7.7%</td>
<td>66.6%</td>
<td>0.863</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>C4d+</td>
<td>6.7%</td>
<td>77.8%</td>
<td>8.9%</td>
<td>&lt;0.001</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NK cells

- CD56+ and CD68+ cells in peritubular capillaries increased in AMR compared to T cell-mediated rejection
  - Effector role for NK cells and macrophages in AMR
- CD16 (FcyR)-inducible NK cell-selective transcripts (CD160 and XCL1) associated with AMR
  - Evidence for Ab-mediated NK cell activation via FcγR (CD16)
  - Raises the possibility of other CD16-triggered effects, including NK localization and cytotoxicity.


NK cells

- Differences in circulating NK cell subsets in transplant patients with DSA, non-DSA alloAb, and no DSA

- Role of CMV?
  - CMV infection promotes an adaptive differentiation and expansion of a subset of mature NK cells


Mast cells

- Non-transplant roles:
  - Fighting parasitic infections
  - Role in IgE-mediated allergic responses
- Mast cells degranulate to release preformed mediators
  - Histamine, heparin, serotonin, and serine proteases

Mast cells

- Non-transplant roles:
  - Fighting parasitic infections
  - Role in IgE-mediated allergic responses
- Mast cells degranulate to release preformed mediators
  - Histamine, heparin, serotonin, and serine proteases
- Mast cells have actions even without degranulating
  - Secrete pro-inflammatory factors (leukotrienes, prostanoids, cytokines)
  - Activate nearby inflammatory cells
  - Recruit other immune cells such as eosinophils, neutrophils

Mast cells

- Seen at sites of chronic inflammation, including nonallergic inflammation
- Mast cells help tissues heal and repair from damage
  - Produce immune-inhibitory cytokine (IL-10) and degrade proinflammatory cytokines with granule proteases
  - Release cytokines (eg, TGF-β) and other factors that change tissue morphology

Mast cells

- Mast cells present in the interstitium; occasional mast cell tubulitis (bx 5 months-5 years, graft dysfunction)
  - Giemsa-stained sections, Epon-embedded

- Mast cell semi-quantitative study in acute rejection
  - Mast cell tryptase-specific monoclonal antibodies, paraffin sections
  - Correlation between number of mast cells and:
    - Time post-transplant
    - Severity of interstitial fibrosis


Mast cells

• 461 consecutive kidney allograft biopsy specimens, CD117 immunostaining to count mast cells
  • Mast cell number correlated with:
    • Banff features of T-cell–mediated and AMR
    • Interstitial fibrosis
    • Time post-transplant
  • Reflective of “cumulative burden of tissue injury”

## Summary

### Non-T cells in the late post-transplant kidney

<table>
<thead>
<tr>
<th>Cell type in kidney</th>
<th>Rejection type</th>
</tr>
</thead>
<tbody>
<tr>
<td>B cells</td>
<td>T cell mediated rejection, combined AMR/TCMR</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>Late combined AMR/ T cell mediated rejection</td>
</tr>
<tr>
<td>Monocytes/macrophages</td>
<td>Late AMR (capillaritis), IFTA+i</td>
</tr>
<tr>
<td>NK cells</td>
<td>Late AMR</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Associated with fibrosis, late T cell mediated rejection</td>
</tr>
</tbody>
</table>
What causes late graft injury and loss?
Conventional view of late allografts

“CAN”, chronic allograft nephropathy
Progressive interstitial fibrosis & tubular atrophy (IFTA), ?due to calcineurin inhibitor (CSA) toxicity

Conventional view of late allografts

Or: progressive antibody-mediated rejection


What do late (10 year) grafts look like histologically?

160 ten year protocol biopsies from conventional kidney transplants performed 2002-2005

92% on tacrolimus-based immunosuppression

Scoring performed using the Banff 2013 system and additional scoring not addressed by Banff
What do late (10 year) grafts look like histologically?

**Biopsies:**
Predominantly glomerular and vascular changes
Mild/no IFTA
Low rate of chronic antibody-mediated rejection
Increased global glomerulosclerosis
Mesangiosclerosis, no diabetes
Peripheral nodular (arrow) and intimal hyalinosis
<table>
<thead>
<tr>
<th>Histologic Lesion</th>
<th>% of biopsies, time 0 (paired, n= 150)</th>
<th>% of biopsies, 5 years (paired, n= 132)</th>
<th>% of biopsies, 10 years (n=160)</th>
<th>p value (5-10 year bx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangiosclerosis (MS)</td>
<td>&lt;1%</td>
<td>31%</td>
<td>65%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glomerulomegaly (GM)</td>
<td>3%</td>
<td>19%</td>
<td>34%</td>
<td>0.0037</td>
</tr>
<tr>
<td>Focal segmental glomerulosclerosis (FSGS)</td>
<td>0%</td>
<td>7%</td>
<td>17%</td>
<td>0.012</td>
</tr>
<tr>
<td>Increased global glomerulosclerosis (GG), &gt;20%</td>
<td>0%</td>
<td>24%</td>
<td>44%</td>
<td>0.011</td>
</tr>
<tr>
<td>Transplant glomerulopathy (TG)</td>
<td>0%</td>
<td>7%</td>
<td>10%</td>
<td>NS (0.40)</td>
</tr>
<tr>
<td>Arteriolar hyalinosis, moderate to severe</td>
<td>0%</td>
<td>18%</td>
<td>65%</td>
<td>0.0001</td>
</tr>
<tr>
<td>Arteriosclerosis, moderate to severe</td>
<td>5%</td>
<td>17%</td>
<td>38%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IFTA, moderate to severe</td>
<td>&lt;1%</td>
<td>9%</td>
<td>12%</td>
<td>NS (0.57)</td>
</tr>
</tbody>
</table>
Histologic/clinical correlates at 10 years

- **Mesangial sclerosis:**
  - Associated with baseline or post-transplant diabetes
  - But still present in ~20% of pts without DM

- **Arteriolar hyalinosis:**
  - More common in diabetics, but still prevalent in patients without DM

- **IFTA:** more common in diabetics
Conclusions from 10 year protocol biopsy data

- Most chronic injury appears to be due to non-immune causes
- Argues for an alternative viewpoint of the mechanism of late graft injury
- From 5 to 10+ years, the graft is confronted with new pathogenic challenges that likely we are not addressing adequately
Thank you!
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