HLA and Non-HLA Antibodies in Transplantation and their Management

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October 29th, 2016
History

• 1960 “donor specific antibodies” (DSA): first suggestion for a possible role in deteriorating renal function
• 1970 (Jeannet) – worse graft outcome when DSA are present
• 1990 (Halloran) - humoral rejection is clearly identified. Clinics and pathology are defined
Hystory II

- 1991, 1993 Feucht identifies “C4d” (byproduct after C4 metabolism) in peritubular capillaries of “high immunonologic risk” patients
- It is then proposed as a specific marker for humoral rejection
Hystory III

• 1999 Collins: C4d staining within peritubular capillaries is associated to circulating antibodies against class I and II HLA donor antigens.
C4d vs donor specific antibodies

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>DSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4d+</td>
<td>20</td>
<td>18 (90%)</td>
</tr>
<tr>
<td>C4d-</td>
<td>47</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

Mauyyedi JASN 2002
Antibody mediated rejection

- Histology
  - acute tubular injury,
  - neutrophils and/or mononuclear cells in peritubular capilaries and/or glomeruli and/or capillary thrombosis, fibrinoid necrosis/intramural or transmural inflammation in arteries
- immunopathologic evidence: C4d or immunoglobulins deposition in peritubular capilaries
- serologic evidence: anti-donor antibodies
The microvasculature of the nephron.
Figure 1: HLA DSA by AMR type at the time of AMR-defining biopsy. AMR, antibody-mediated rejection.

P = 0.4
<table>
<thead>
<tr>
<th>AMR Status</th>
<th>Hazard Ratio (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4d-negative AMR</td>
<td>2.56 (1.08–6.05)</td>
<td>0.033</td>
</tr>
<tr>
<td>C4d-positive AMR</td>
<td>3.70 (2.47–5.54)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
DONOR SPECIFIC ANTIBODIES
question

• are all donor antibodies directed against HLA antigens?
Antibody mediated rejection

- Preformed / de novo antibodies
  Against class I or II anti HLA antigens
  Ab vs Non-HLA antigens:
  - MICA: Major-histocompatibility-complex class I–related chain A antigens
  - AT\textsubscript{1}R-AA: Agonistic antibodies against the Angiotensin II type 1 receptor
  - Others (Anti-endotheline type 1 receptor, antiperlecan antibodies,....)
What are MICA?

• MICA = Major-histocompatibility-complex class I–related chain A (MICA) antigens
• are surface glycoproteins with functions related to innate immunity.
• are expressed on endothelial cells, dendritic cells, fibroblasts, epithelial cells, but not on peripheral-blood lymphocytes.
• Therefore, antibodies directed against MICA are not detected with the methods generally used for cross-match.

Agonistic antibodies against the Angiotensin II type 1 receptor (AT1R-AA)

- Classically reported a rejection with severe hypertension
- Hystology: endarteritis, transmural arteritis and/or fibrinoid vascular necrosis (Banff IIb or Banff III rejection)
- Is it a “true-rejection” or an autoimmune phenomenon triggered in the permissive allogeneic and post-ischemic inflammatory enviroment?

TREATMENT
Therapeutic Approaches For Crossing Antibody Barriers to Solid Organ Transplantation

- B-cells & Pre B-cells
- Clonal Expansion
  - Anti-CD20
  - Splenectomy

- Plasma cells
  - Bortezomib
- Allo-reactive Antibodies
  - Plasmapheresis/IVlg

- Complement Activation and Endothelial Destruction
  - Anti-C5
Immunoglobulin

- 20 highly sensitized patients (PRA 77±19%) were enrolled and received treatment with intravenous immune globulin and rituximab
- 16/20 received a transplant.
- At 12 months, the mean serum creatinine level was 1.5±1.1 mg/dl (133±97 μmol/l)
- mean survival rates of patients and grafts were 100% and 94%, respectively

Immunoglobulin

- double-blind placebo controlled trial of high-dose IVIg-based desensitization
- compared high dose IVIg alone vs. high-dose IVIg plus rituximab in patients with PRA > 80% (clinicaltrials.gov study #NCT01178216; 42).
- IVIg (2 g/kg weeks 1 and 4) and rituximab
- (1 g given at week 2).
- The trial was originally designed to enroll 90 patients, but was halted by the DSMB after only 15 patients were enrolled because of high AMR and allograft loss rates.

Am J Transplant 2013: 13(Suppl 5): 76 abstract #153
Immunoglobulin

• Two additional study have not been able to reproduce the potential of immunoglobulin in reducing anti-HLA antibody levels and improving transplantation rates, specifically in patients with PRA >80%

  Transplantation 2012; 94: 345–351
Rituximab is a chimeric antibody recognizing the cell surface marker CD20, which is expressed at most stages of B-cell development except the very early stages, but not on plasma cells.
IVIG and rituximab: pediatric patients

### Rituximab and desensitization: review

**Table:**

<table>
<thead>
<tr>
<th>Study (yr) country</th>
<th>No. of patients (RTX/non-RTX)</th>
<th>Study period, mo</th>
<th>Treatment regimen (RTX/non-RTX)</th>
<th>Baseline IS</th>
<th>T-cell induction therapy</th>
<th>Patient survival</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Retrospective cohort studies</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyodo (2011)* Japan (34)</td>
<td>122 (29/31/62)</td>
<td>60</td>
<td>RTX+MMF/SPX+MMF/SPX+AZA</td>
<td>Not fully reported</td>
<td>Not reported</td>
<td>Not reported</td>
</tr>
<tr>
<td>Aikawa (2011)* Japan (35)</td>
<td>111 (16/95)</td>
<td>36</td>
<td>RTX+PE or PP/SPX+PE or PP</td>
<td>TAC or CsA, MMF or AZA+CS</td>
<td>BXM(^c)</td>
<td>No difference</td>
</tr>
<tr>
<td>Tanabe (2007) Japan (17–21, 36–41)</td>
<td>102 (57/45)</td>
<td>24</td>
<td>RTX+PP/SPX+PP</td>
<td>TAC, MMF+CS</td>
<td>BXM</td>
<td>No difference(^d)</td>
</tr>
<tr>
<td>Ashimine (2014) Japan (22)</td>
<td>81 (30/51)</td>
<td>36</td>
<td>RTX+PP/SPX+PP</td>
<td>TAC or CsA+MMF or MZR</td>
<td>BXM</td>
<td>No statistical comparison</td>
</tr>
<tr>
<td>Harada (2013)* Japan (42)</td>
<td>70 (46/24)</td>
<td>60</td>
<td>RTX+PP/SPX+PP</td>
<td>TAC, MMF, or AZA+CS</td>
<td>BXM or ALG</td>
<td>No statistical comparison</td>
</tr>
<tr>
<td>Charif (2013)* UK (43)</td>
<td>63 (24/39)</td>
<td>36</td>
<td>RTX+PE/ALZ+PE</td>
<td>TAC+CS±MMF(^g)</td>
<td>DAC (RTX group only)</td>
<td>No difference</td>
</tr>
<tr>
<td>Nakagawa (2011)* Japan (44)</td>
<td>61 (42/19)</td>
<td>36</td>
<td>RTX/SPX</td>
<td>TAC or CsA, MMF+CS(^h)</td>
<td>BXM (RTX group only)</td>
<td>No difference</td>
</tr>
<tr>
<td>Montgomery (2009) USA (23)</td>
<td>60 (3/15/14/28)</td>
<td>60</td>
<td>RTX, iVig, PP+SPX/RTX, iVig+PP/SPX, iVig+PP/</td>
<td>TAC, MMF+CS</td>
<td>DAC</td>
<td>Not reported</td>
</tr>
<tr>
<td>Gloor (2005) USA (24)</td>
<td>34 (11/23)</td>
<td>24</td>
<td>RTX, iVig+PP/SPX, iVig+PP</td>
<td>TAC, MMF+CS</td>
<td>ATG</td>
<td>No difference</td>
</tr>
<tr>
<td>Waigankar (2013) India (25)</td>
<td>26 (7/19)</td>
<td>12–18</td>
<td>RTX, PP+iVig/SPX, PP+iVig</td>
<td>TAC, MMF+CS</td>
<td>Not reported</td>
<td>No statistical comparison</td>
</tr>
</tbody>
</table>

*Transplantation 2014;98: 794-805*
Rituximab: conclusions

• no strong evidence exists to support superior patient and graft outcomes with rituximab
• optimal dose and number of infusions of rituximab is still unknown
• the diversity of therapeutic protocols, using a variety of complex medications, means that it is difficult to confidently attribute outcomes solely to the administration of rituximab

Transplantation 2014;98: 794-805
One-year Results of the Effects of Rituximab on AMR

ALL: PE/3 CS pulses +
maintenance: steroids + tacrolimus (TL 8-12 ng/mL) + MMF (2 g/day)

40 patients randomized

Placebo group: 19 patients
  Intention to treat: 19 patients
    1 patient received Rituximab
      First rescue injection: 8 patients

Rituximab group: 21 patients
  Intention to treat: 19 patients
    1 patient received Placebo
      First rescue injection: 6 patients

Treatment not performed: 2 patients

Per protocol analysis:
  Only Placebo during the study: 11 patients
  At least one Rituximab injection during the study: 27 patients

Transplantation 2016;100: 391–399
Rituximab: infections

- None of the studies found a statistically significant higher incidence infectious of complications with rituximab. Indeed, significantly lower rates of CMV viremia and viral infections were identified, possibly for a lower number of episodes of rejection and associated steroid therapy (Transplantation 2014;98: 794-805).

- Other reports suggest that desensitization with rituximab and IVIg may result in a greater incidence of BKV viremia after transplantation (Am J Transplant 2009; 9: 244, Transplantation 2014;97: 755-761).
Alemtuzumab

Lymphocyte-depleting, CD52-specific, monoclonal antibody: conflicting results
Alemtuzumab

- Potential negative effects of alemtuzumab on the regulation of humoral immunity, possibly due to dysregulation of B cell activating factor (BAFF), as an increase in BAFF mRNA expression include:
  - unexpectedly high rates of ABMR
  - high rates of circulating alloantibody
  - intragraft C4d at 1-year posttransplant

bortezomib

- Bortezomib is a proteasome inhibitor that acts on plasma cells and is effective in removing preformed DSA when combined with plasmapheresis.
- It is also associated with durable reductions in DSA and stable allograft function in de novo DSA-positive renal transplant recipients.

Prospective iterative trial:

- 44 sensitized patients treated – 19 transplanted
- **median follow-up of 436 days**
- Acute rejection rates: 18.8%
- De novo DSA formation (12.5%).
- Patient and graft survival were 100% and 94.7%

eculizumab

- 26 hyperimmune patients were treated with eculizumab post-transplantation vs 51 historical controls
- Both groups were treated pretransplantation with plasmaexchange (PE)
- After transplantation only control patients were treated by means of PE

*Am J Transpl 2011; 11: 2405–2413*
Results

• incidence of AMR was 7.7% (2/26) in the eculizumab group compared to 41.2% (21/51) in the control group (p = 0.0031)

• On 1-year protocol biopsy, transplant glomerulopathy was found to be present in 6.7% (1/15) eculizumab-treated recipients and in 35.7% (15/42) of control patients (p = 0.044)

CONCLUSION: eculizumab decreases the incidence of early AMR in sensitized renal transplant recipients

*Am J Transpl 2011; 11: 2405–2413*
BUT ... long term Results

CONCLUSION: despite decreasing acute clinical ABMR rates, EC treatment does not prevent chronic ABMR in recipients with persistently high BFXM after XMKTx.

Am J Transplant. 2015;15:1293-302
C1 Inhibition

• C1 inhibitor (C1-INH) is a multifunctional member of the serpin family of protease inhibitors. C1-INH inactivates both C1r and C1s and is the only plasma protease that regulates the classic complement pathway.

• All patients with PRA > 50 % treated with rituximab + IgG

• 10 pts treated with C1-INH and 10 with placebo

• Primary end point: ABMR at 6 months

Transplantation 2015;99: 299–308
C1 Inhibition: results

- No significant difference was seen in rejection rate between treated and non treated patients
- in vitro experiments revealed that C1-INH was very efficient at inhibiting C1q binding to luminex beads induced by low-titer HLA antibodies and less effective with high-titer antibodies

Transplantation 2015;99: 299–308
6 patients were treated
Sensitized patients

DSA removal (immunoadsorption or plasma exchange), DSA inactivation (high-dose intravenous immunoglobulins) enable successful positive-crossmatch kidney transplantation with good short- to intermediate term outcomes.

*Nat. Rev. Nephrol.* 6, 297–306 (2010);
However:

Antibody-mediated rejection can occur subclinically and in time results in chronic injury to the renal microvasculature, transplant glomerulopathy, interstitial fibrosis, and tubular atrophy

*Nat. Rev. Nephrol. 6, 297–306 (2010)*;
and

- acute antibody mediated rejection (AMR) occurs in 20–50% of positive crossmatch transplantations.
- AMR is usually reversed: 1 year survival close to 90%
- but 3, 5 or 8 years survival significantly worse than “standard”

*Nat. Rev. Nephrol. 6, 297–306 (2010);*
Graft survival in positive cross-match cases compared with controls

Am J Transpl 2009; 9: 536–542
IS THERE (ALREADY) A ROLE FOR MESENCHIMAL STEM CELLS?
Table 2 | Clinical studies of BM-derived MSCs in kidney transplantation

<table>
<thead>
<tr>
<th>Study</th>
<th>Induction therapy (dose)</th>
<th>Maintenance immunsuppression</th>
<th>No. of patients</th>
<th>MSC Source</th>
<th>Dose (cells per kg x 10^6)</th>
<th>Timing</th>
<th>Main finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perico et al. (2011)^29</td>
<td>rATG (0.5 mg, day 0–6); basiliximab (20 mg days 0 and 4); steroids (day 0 to 7)</td>
<td>CsA, MMF</td>
<td>2</td>
<td>Autologous</td>
<td>1.7–2.0</td>
<td>Day 7</td>
<td>Increased T&lt;sub&gt;REG&lt;/sub&gt; cell:memory CD8 T cell ratio from baseline; engraftment syndrome in two patients</td>
</tr>
<tr>
<td>Perico et al. (2013)^100</td>
<td>rATG (0.5 mg, day 0–6); steroids (day 0–7)</td>
<td>CsA, MMF</td>
<td>2</td>
<td>Autologous</td>
<td>2.0</td>
<td>Day 1</td>
<td>Increased T&lt;sub&gt;REG&lt;/sub&gt; cell:memory CD8 T cell ratio from baseline; acute cellular rejection in one patient</td>
</tr>
<tr>
<td>Tan et al. (2012)^101</td>
<td>Basiliximab in control group only (20 mg, days 0 and 4)</td>
<td>CNI, MMF, steroids</td>
<td>105 (53 on standard CNI dose; 52 on 80% CNI dose)</td>
<td>Autologous</td>
<td>1.0–2.0</td>
<td>Day 0 and 14</td>
<td>Reduced incidence of acute rejection at 6 months and lower incidence of viral infections in the MSC group than in the control group</td>
</tr>
<tr>
<td>Reinders et al. (2013)^102</td>
<td>Basiliximab (20 mg, day 0 and 4)</td>
<td>CNI, MMF, steroids</td>
<td>6</td>
<td>Autologous</td>
<td>1.0–2.0 (two doses 7 days apart)</td>
<td>Week 4 or month 6</td>
<td>MSC infusion enabled resolution of tubulitis and IFTA in two patients with subclinical rejection; opportunistic viral infection in three patients</td>
</tr>
<tr>
<td>Mudrabettu et al. (2015)^103</td>
<td>rATG (1mg/kg, day -1 to +1)</td>
<td>Tacrolimus, MMF, steroids</td>
<td>4</td>
<td>Autologous</td>
<td>0.2–0.8</td>
<td>Day 1 and 30</td>
<td>No early or late kidney graft dysfunction and no viral infections in the KTRs</td>
</tr>
<tr>
<td>Peng et al. (2013)^104</td>
<td>Cytoxan (200 mg)</td>
<td>Tacrolimus, MMF, steroids</td>
<td>6</td>
<td>Donor</td>
<td>5.0 (renal artery at day 0) and 0.2 (IV day 30)</td>
<td>Day 0 and 30</td>
<td>50% reduction of tacrolimus dose in the MSC group</td>
</tr>
</tbody>
</table>

BM, bone marrow; CNI, calcineurin inhibitor (CsA or tacrolimus); CsA, ciclosporin A; IFTA, interstitial fibrosis and tubular atrophy; IV, intravenous; KTR, kidney transplant recipient; MMF, mycophenolate mofetil; MSC, mesenchymal stromal cell; rATG, rabbit anti-thymocyte globulin; T<sub>REG</sub>, T regulatory.
Table 1 Registered clinical trials of mesenchymal stem cells in kidney transplantation (ClinicalTrials.gov, updated July 2015)

<table>
<thead>
<tr>
<th>NCT</th>
<th>Status</th>
<th>Title</th>
<th>Site</th>
<th>Type of MSC</th>
<th>Start date</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT02409940</td>
<td>Recruiting</td>
<td>To elucidate the effect of mesenchymal stem cells on the T-cell repertoire of kidney transplant patients</td>
<td>Chandigarh, India</td>
<td>Autologous/allogeneic; BM-MSC</td>
<td>September 2013</td>
</tr>
<tr>
<td>NCT02387151</td>
<td>Recruiting</td>
<td>Allogeneic mesenchymal stromal cell therapy in renal transplant recipients</td>
<td>Leiden, Netherlands</td>
<td>Allogeneic; BM-MSC</td>
<td>March 2015</td>
</tr>
<tr>
<td>NCT02057965</td>
<td>Recruiting</td>
<td>Mesenchymal stromal cell therapy in renal recipients</td>
<td>Leiden, Netherlands</td>
<td>Autologous; BM-MSC</td>
<td>March 2014</td>
</tr>
<tr>
<td>NCT02012153</td>
<td>Recruiting</td>
<td>Mesenchymal stromal cells in kidney transplant recipients</td>
<td>Bergamo, Italy</td>
<td>Autologous; BM-MSC</td>
<td>December 2013</td>
</tr>
<tr>
<td>NCT00659620</td>
<td>Unknown</td>
<td>Mesenchymal stem cell transplantation in the treatment of chronic allograft nephropathy</td>
<td>Fuzhou, Fujian</td>
<td>Autologous; BM-MSC</td>
<td>May 2008</td>
</tr>
<tr>
<td>NCT00734396</td>
<td>Completed</td>
<td>Mesenchymal stem cells and subclinical rejection</td>
<td>Leiden, Netherlands</td>
<td>Autologous; BM-MSC</td>
<td>February 2009</td>
</tr>
<tr>
<td>NCT00752479</td>
<td>Terminated</td>
<td>Mesenchymal stem cells under basiliximab/low dose RATG to induce renal transplant tolerance</td>
<td>Bergamo, Italy</td>
<td>Autologous; BM-MSC</td>
<td>May 2008</td>
</tr>
<tr>
<td>NCT00658073</td>
<td>Completed</td>
<td>Induction therapy with autologous mesenchymal stem cells for kidney allografts</td>
<td>Fuzhou, Fujian</td>
<td>Autologous; BM-MSC</td>
<td>March 2008</td>
</tr>
<tr>
<td>NCT01429038</td>
<td>Recruiting</td>
<td>Mesenchymal stem cells after renal or liver transplantation</td>
<td>Liege, Belgium</td>
<td>Allogeneic; BM-MSC</td>
<td>February 2012</td>
</tr>
</tbody>
</table>

BM-MSC bone marrow-derived mesenchymal stem cell, MSC mesenchymal stem cell, NCT ClinicalTrials.gov identifier, RATG rabbit antithymocyte globulin
Non-HLA antibodies

Pharmacologic antagonists targeting the ETAR (sentanes)?

Losartan

Figure 1 | Overview of nonhuman leukocyte antigen antibodies directed against endothelial targets. AT₁R, angiotensin II type 1 receptor; ET₄R, endothelin-1 type A receptor; FLT3, Fms-like tyrosine kinase-3; ICAM4, intercellular adhesion molecule 4.

Kidney Int. 2016; 90:280-8
Conclusions I

- Donor Specific Antibodies worsen graft outcome
- They may be directed toward several different antigens
Conclusions II

- Current therapies, including
  - DSA removal (plasma exchange/immunoadsorption)
  - DSA modulation (intravenous immunoglobulin ± rituximab)
  - complement component antagonists (eculizumab)

  have been relatively successful to treat **acute** AMR.

- In contrast, chronic progression in AMR has proven to be intractable so far
Conclusions III

early identification of non HLA antibodies could lead to timely initiation of possibly effective targeted therapies