Il danno da Ischemia Riperfusione
Tissue injury in kidney Transplantation

- Warm ischemia
- Cold ischemia
- Reperfusion injury
- Immune rejection and drug toxicity
- Chronic Organ Failure

Preservation Drug
I/R Drug
Anti-rejection Drug
Emerging Therapies Targeting Intra-Organ Inflammation in Transplantation

American Journal of Transplantation
Volume 15, Issue 2, pages 305-311, 22 JAN 2015 DOI: 10.1111/ajt.13073
http://onlinelibrary.wiley.com/doi/10.1111/ajt.13073/full#ajt13073-fig-0001

Solhjlou 2015
Consequences of donor organ ischemia on innate immune activation
Pattern recognition receptors expressed throughout the nephron
Inflammasome signaling in AKI
Maladaptive repair following acute kidney injury (AKI)
Deceased Donor Kidney Transplantation: A Model of Ischemia-Reperfusion Injury

Potential Deceased Donor
Hypotension, shock, acute inflammation, kidney injury

Brain death or DCD

Ex vivo
Organ preservation – Hypothermia and hypoxia

Recipient
Vascular anastomosis with re-perfusion injury
Experimental strategies for identifying renoprotective approaches for AKI: from rodent to mammalian models.

Zheng Dong 2016
Experimental strategies for identifying renoprotective approaches for AKI: from single to multiple models.
Role of complement in renal ischemia-reperfusion injury, inflammation, and progression to kidney fibrosis.

Fernandez 2014
Multifaceted activity of the complement system in immunity.

Fernandez 2014
Complement system: a biochemical cascade made up of approximately 30 serum and membrane-bound proteins.

- The primary location for biosynthesis of complement is the liver.
- The extrahepatic complement synthesis contributes approximately 10% of circulating C3. The alternative sites for complement production include epithelial cells, fibroblasts, lymphocytes and macrophages derived from different organs, including the kidney.
Complement System 2/2

- Complement activation initiates a cascade reaction, which leads to the cleavage of inert plasmatic components that generate bioactive components, including C3b, C3a, C5a, and C5b-9, with pro-inflammatory, chemo-attractant, and cell-damaging functions.

- A set of at least seven proteins in plasma (C1 INH, C4b-binding protein, factor H, and factor I) or cell membranes (decay-accelerating factor, membrane cofactor protein, and CR1 (CD35)) modulate the complement proteins and protect host cells and tissues from complement damage.
Targeting the complement cascade: novel treatments coming down the pike

Joshua M. Thurman, Moglie Le Quintrec

http://dx.doi.org/10.1016/j.kint.2016.04.018
The complement system and kidney disease

Joshua M. Thurman, Moglie Le Quintrec

Targeting the complement cascade: novel treatments coming down the pike

The complement system and kidney disease

Afferent vessel
- Inflow of complement proteins and fragments

Mesangium
- Mesangial immune-complexes
- Antibodies specific for antigens expressed in mesangial cells

Capillary wall
- Subendothelial immune-complexes
- Subepithelial immune-complexes
- Antibodies specific for antigens expressed in the GBM, podocytes, or mesangial cells.
- No intrinsic complement regulatory proteins expressed within GBM
- Loss of CR1 from podocytes

Proximal tubule
- No complement regulators expressed on apical surface
- Local production of complement proteins
- Local production of ammonia
- Reduced complement regulators on basolateral surface after injury
Local Renal Synthesis of Complement

The first study to demonstrate that human renal proximal tubular epithelial cells synthesised and secreted complement component C3 in vitro was published by Brooimans et al. more than 20 years ago.

Resident renal cells, including tubular and glomerular epithelial cells, mesangial cells and endothelial cells can synthesise many, if not all complement proteins.
Local synthesis of complement component C3 regulates acute renal transplant rejection

JULIAN R. PRATT, SHAMIM A. BASHEER & STEVEN H. SACKS

Department of Nephrology & Transplantation, King's College University of London,
Guy's Hospital, London, UK
Correspondence should be addressed to S.H.S.; email: steven.sacks@kcl.ac.uk

NATURE MEDICINE • VOLUME 8 • NUMBER 6 • JUNE 2002
Local Synthesis of Complement System induces Acute Rejection (2)

Experimental Design:

1. **DONOR**
   - WT
   - **RECIPIENT**
   - WT
   - **GRAFT SURVIVAL**
   - After 21 days: 100% loss (Mean: 12.5 days)

2. **DONOR**
   - C3 -/-
   - **RECIPIENT**
   - WT
   - **GRAFT SURVIVAL**
   - After 100 days: 20% loss (at day 67 & 85)

3. **DONOR**
   - WT
   - **RECIPIENT**
   - C3 -/-
   - **GRAFT SURVIVAL**
   - After 24 days: 100% loss (Mean: 16.2 days)
Currently, there are 2 FDA approved drugs aimed at inhibition of complement activation:

- Eculizumab (anti-C5) which is approved for the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome.

- C1-Inhibitor (C1-INH), which is approved for use in patients with hereditary angioedema
One of the cardinal features of AMR is C4d (a fragment of C4 which remains on the target after C activation). C1-INH inactivates both C1r and C1s and is the only plasma protease that regulates the classic complement pathway thus preventing proteolytic activation of C4 and C2 that form C3 convertase.

C1-INH can also inhibit the analogous serine proteases in the lectin pathway of C activation, as well as the alternative pathway through regulation of factor B binding to C3b as well as factor B cleavage by factor D.

C1-INH also has major effects on activation of the coagulation cascade and on regulation of vascular permeability and inflammation by kinins.
Complement in immune-mediated renal disease

1. Brain death / Cardiac Arrest
2. Ischemia / Reperfusion Injury
3. Lupus Nephritis
4. IgAN
4. aHUS
Brain death (BD) is complex and causes a systemic and local inflammatory response and resembles the systemic inflammatory response syndrome (SIRS).

The cause of this immune activation is not well understood.

Kidney from brain-dead organ donors give inferior results compared to kidneys from living donors:

<table>
<thead>
<tr>
<th>Organ transplanted</th>
<th>1-year survival</th>
<th>5-year survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Living donor</td>
<td>94.3%</td>
<td>78.6%</td>
</tr>
<tr>
<td>Deceased donor</td>
<td>88.7%</td>
<td>65.7%</td>
</tr>
</tbody>
</table>

Source: UNOS/OPTN.
The complement system in brain death

Local complement production by the kidney

Renal C3 expression in rats

Renal C3 expression in humans


Renal expression of complement in the donor graft

Components of the CP

The role of C5a in brain-dead donors

Plasma C5a levels

Renal C5a-Receptor expression

The effect of renal C5a-C5aReceptor activation

Complement inhibition in brain-dead donors

**Animal model:**
Rat Brain death with Renal transplantation.

**Intervention:**
sCR1 = C3 inhibitor (soluble Complement Receptor 1)

**Treatment:**
- Before BD induction
- After BD induction

**Conclusion:**
Complement inhibition improves renal function after Tx in the recipient.

Ischemia/Reperfusion injury (IRI) in kidney transplantation

IRI is a frequent event in kidney transplantation, particularly when the kidney comes from a deceased donor, and can heavily influence both the early and the late function of a kidney allograft.

Renal Allograft

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm Ischemia Time (WIT)</td>
<td>≤ 60 minutes</td>
</tr>
<tr>
<td>Cold Ischemia Time (CIT)</td>
<td>≤ 72 hours</td>
</tr>
<tr>
<td>(≤24 hours ideal)</td>
<td></td>
</tr>
</tbody>
</table>
The Lectin Pathway in IRI

- The recognition molecules of the MBL pathway include mannose binding lectins or ficolins. These molecules bind to carbohydrate motifs displayed on the surface of bacteria, but MBL could bind neo-epitopes that are generated or exposed within the injured kidney (i.e. Cytokeratin)

- Patients with high circulating concentrations of MBL have a poor outcome after renal transplantation.
  Berger SP, AJP 2005

- Animal model
  Transgenic mice deficient in both MBL-A and MBL-C, renal I/R injury

- Results
  - Level of BUN and Creatinine showed that MBL double knock-out mice were protected by IRI, compared to WT mice.

  - The protective phenotype was reversed following reconstitution of the mutant mice with recombinant MBL.

Moller-Kristensen et al., 2005
Ricklin D, Nat Rev 2016
Swine model of IRI

Recombinant Human C1-INH (Ruconest, Pharming, Leiden, The Netherlands)

- Large White Pigs (n=5 per group)
- Induction of ischemia
- Reperfusion
- Biopsies (minutes from reperfusion)
- T0, 30 min, T15, T30, T60, T24

Therapeutic targeting of classical and lectin pathways of complement protects from ischemia-reperfusion-induced renal damage.


C1-INH inhibits C4d deposition

Castellano et al, Am J Pathol, 2010
C4d deposits on renal Endothelial Cells
C1-INH treatment significantly reduced the numbers of inflammatory infiltrating cells
C1-INH infusion modulates NADPH activity and NOX-4 and NOX-2 expression

Complement-mediated endothelial dysfunction in I/R injury

Curci C., Castellano G. Nephrol Dial Transplant. 2014
Endothelial to Mesenchimal Transition in AKI

Stasi et al, NDT 2016
Emerging role of Colec11 in IRI

IRI: Absence of CL-11 in Colec11–/– mice permitted a less severe loss of renal function, with good preservation of renal architecture (Fig A, PAS staining) reduced leukocyte infiltration and tubular deposition of complement compared with that seen in Colec11+/+ mice.

Colec11+/+ mice transplanted with kidneys from Colec11+/+ or Colec11–/–: Colec11–/– transplants showed strong protection from renal tubular damage compared with that observed in Colec11+/+ control transplants.

Farrar CA, Sacks SH, JCI 2016
Role of Complement in renal IRI

Danobeitia et al. Fibrogenesis & Tissue Repair 2014
Complement is bad for aging

- Augmented Wnt signaling is implicated in mammalian aging and aging-related phenotypes and fibrosis
- Serum C1q levels increase with aging
- The C1q complement protein is an activator of canonical Wnt signaling
- C1q-dependent Wnt signaling impairs the regenerative capacity of skeletal muscles
Kidney transplantation is the best option for patients with ESKD;

Despite improvements in first-year graft survival, long-term failure of kidney transplants remains an important clinical problem;

There are several immunological and non-immunological factors related to renal graft deterioration, however several histological lesions of chronic rejection and allograft nephropathy overlap with those observed in aging kidneys.

---

‘Originally described in human fibroblasts by Hayflick and Moorhead, ’ Cellular senescence’ describes a phenotype of permanent and irreversible growth arrest shown by mammalian cells.

Main mechanism are:

• Telomere shortening
• Cell cycle Inhibition

- A telomere is a region of repetitive nucleotide sequences at each end of a chromosome, which protects the end of the chromosome from degradation
- In humans, average telomere length declines from about 11kb at birth to less than 4 kb in old age
- In aging human kidney telomere shortening is faster in cortex compared to medulla (Melk A, JASN, 2000)
A defect in Klotho expression in mice leads to a premature-aging syndrome.

Overexpression of Klotho extends life span in mice (by 20 and 30%)
Klotho: anti-aging gene

- α-Klotho is **mainly expressed in renal tubular cells**
- Membrane form regulates mineral homeostasis
- Soluble form, present in blood, urine and cerebrospinal fluid, acts as an endocrine factor interfering with renal and extrarenal functions

*Hu et al. Nephrol Dial Transplant 2012*

*Wang Y. Ageing Res Rev 2009*
Klotho modulation in IRI

Large White Pigs (n=5 per group)

Induction of Ischemia

Reperfusion

T0

30 min

T24

Sacrifice

T0

T24

50.00 um

50.00 um

C1-INH treatment preserved renal Klotho

Injections C1-INH (C1-INH group) or saline solution (CTR group)

Induction of ischemia

Reperfusion

T0 30 min 24h

T24

Sacrifice

Large White Pigs (n=5 per group)

Ctr T24

C1-Inh T24

Klotho down-regulation in transplant recipients with DGF

serum Klotho in DGF vs EGF patients at 2 years post-Transplant

CELLULAR SENESCENCE: Inflammaging

Post-operative stress, inflammation, antibody mediated Rejection, Nephropathies, Diabetes

SASP (Senescence-Associated Secretory Phenotype)
- increase IL-6, MCP-1, IL-1β
- decrease EGF, IGF-1

- Telomere shortening
- Cell Cycle Inhibition

p16INK4a protein (gene CDKN2A) inhibits the activity of the cyclin-dependent kinases 4 and 6, leading to hypophosphorylation of the retinoblastoma gene and irreversible cell-cycle arrest.

The tubular epithelial cells are most sensitive for the induction of senescence. [Joosten SA, KI 2004]

Ferenbach, D. A. & Bonventre, J. V. Nat. Rev. Nephrol. 11, 264–276 (2015);
Figure 1 Innovative treatments at the donor, graft preservation or recipient levels to improve kidney recovery

Bon, D. et al. (2012) New strategies to optimize kidney recovery and preservation in transplantation
Controllando i livelli plasmatici di mediatori infiammatori, si potrebbero minimizzare i danni d’organo

First report of cytokine removal using CytoSorb® in severe noninfectious inflammatory syndrome after liver transplantation

Dana R. Tomescu1, Simona Olimpia Dima2, Daniela Ungureanu4, Mihai Popescu2, Dan Tulbure24, Irinel Popescu1,2

<table>
<thead>
<tr>
<th></th>
<th>GM-CSF</th>
<th>IFNγ</th>
<th>IL-1β</th>
<th>IL-2</th>
<th>IL-4</th>
<th>IL-5</th>
<th>IL-6</th>
<th>IL-7</th>
<th>IL-8</th>
<th>IL-10</th>
<th>IL-12p70</th>
<th>IL-13</th>
<th>MCP-1</th>
<th>TNFα</th>
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<tbody>
<tr>
<td>T1</td>
<td>7,55</td>
<td>0,50</td>
<td>8,73</td>
<td>30,53</td>
<td>14,37</td>
<td>&lt;2,00</td>
<td>223,18</td>
<td>51,31</td>
<td>210,69</td>
<td>188,95</td>
<td>14,43</td>
<td>19,22</td>
<td>1963,67</td>
<td>45,49</td>
</tr>
<tr>
<td>T2</td>
<td>7,55</td>
<td>&lt;2,49</td>
<td>8,96</td>
<td>37,81</td>
<td>12,66</td>
<td>&lt;2,00</td>
<td>89,99</td>
<td>52,20</td>
<td>297,74</td>
<td>113,74</td>
<td>12,95</td>
<td>19,22</td>
<td>2369,63</td>
<td>49,70</td>
</tr>
<tr>
<td>T3</td>
<td>7,20</td>
<td>&lt;2,49</td>
<td>6,62</td>
<td>40,35</td>
<td>13,23</td>
<td>&lt;2,00</td>
<td>75,65</td>
<td>51,31</td>
<td>53,22</td>
<td>17,54</td>
<td>14,18</td>
<td>18,84</td>
<td>257,07</td>
<td>35,13</td>
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<tr>
<td>T4</td>
<td>5,22</td>
<td>&lt;2,49</td>
<td>2,39</td>
<td>22,27</td>
<td>14,37</td>
<td>&lt;2,00</td>
<td>56,95</td>
<td>51,31</td>
<td>119,90</td>
<td>61,34</td>
<td>12,95</td>
<td>18,45</td>
<td>509,99</td>
<td>62,71</td>
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<tr>
<td>T5</td>
<td>6,19</td>
<td>6,80</td>
<td>7,79</td>
<td>22,43</td>
<td>21,25</td>
<td>12,94</td>
<td>31,81</td>
<td>51,31</td>
<td>299,89</td>
<td>48,65</td>
<td>13,68</td>
<td>18,84</td>
<td>399,14</td>
<td>64,65</td>
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<tr>
<td>Mean</td>
<td>5,38</td>
<td>0,50</td>
<td>2,02</td>
<td>21,81</td>
<td>13,92</td>
<td>12,94</td>
<td>7,25</td>
<td>25,54</td>
<td>20,20</td>
<td>14,23</td>
<td>12,75</td>
<td>18,07</td>
<td>281,61</td>
<td>16,09</td>
</tr>
</tbody>
</table>
Rimozione delle citochine In Vivo

* Riduzione significativa di IL-6 e IL-10 (p<.05) in 8 h di emoperfusione con Cytosorb

Ridotta produzione di Citochine In Vivo

CytoSorb™ riduce sostanze, tra cui NF-κB, che determina la produzione di citochine.

Rimozione PAMPS & DAMPS In Vitro

*In vitro* adsorption of a broad spectrum of sepsis inflammatory mediators with CytoSorb® hemoadsorbent polymer beads

---

**In Vitro Adsorption of DAMPS from Blood with CytoSorb® or Control Device**

- S100-A8: Control
- Procalcitonin: Control
- C5a: Control
- S100-A8: CytoSorb
- Procalcitonin: CytoSorb
- C5a: CytoSorb

**In Vitro Adsorption of Cytokines**

- TNF-α: Control
- HMGB: Control
- IL-8: Control
- TNF-α: CytoSorb
- HMGB-1: CytoSorb
- IL-8: CytoSorb

**In Vitro Adsorption of PAMPS from Blood with CytoSorb® or Control Device**

- α-Toxin: Control
- SPE B: Control
- TSST: Control
- Afatoxin: Control
- α-Toxin: CytoSorb
- SPE B: CytoSorb
- TSST-1: CytoSorb
- Afatoxin: CytoSorb

Gruda et al. Crit Care 2016, Suppl
<table>
<thead>
<tr>
<th>Cytokines</th>
<th>DAMPS</th>
<th>PAMPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP1-α</td>
<td>Complement (C5a, C3a)</td>
<td><strong>Bacterial Exotoxins:</strong></td>
</tr>
<tr>
<td>IL-6</td>
<td>S100 proteins (S100A8, S100A9)</td>
<td>• Pneumolysin, Streptolysin</td>
</tr>
<tr>
<td>IL-8</td>
<td>Procalcitonin</td>
<td>• SPE B</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>HMG-B1</td>
<td>• TSST-1</td>
</tr>
<tr>
<td>TNF-α</td>
<td></td>
<td>• STX-1, STX-2</td>
</tr>
</tbody>
</table>

**Metabolites**

- Pancreatic Trypsin,
  Chymotrypsin
- Free hemoglobin, myoglobin
- Bilirubin

**PAMPS**

- Hemolysins:
  - *Staph aureus α-toxin*
- Mycotoxins
  - *Aflatoxin*
Table 1 Characteristics of current preservation solutions

<table>
<thead>
<tr>
<th>Solution type</th>
<th>K⁺ (mM)</th>
<th>Na⁺ (mM)</th>
<th>Buffer (pH)</th>
<th>Impermeants</th>
<th>Adenosine (mM)</th>
<th>Antioxidants</th>
<th>Colloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracellular type solution [K⁺] &gt; 62 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Belzer UW® (ViaSpan™)</td>
<td>125</td>
<td>30</td>
<td>Phosphate (7.3)</td>
<td>+</td>
<td>5</td>
<td>+</td>
<td>HES (50 g/l)</td>
</tr>
<tr>
<td>Intermediate type solution [K⁺] 7–62 mM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGL-1®</td>
<td>30</td>
<td>125</td>
<td>Phosphate (7.3)</td>
<td>+</td>
<td>5</td>
<td>+</td>
<td>PEG 35 kDa (1 g/l)</td>
</tr>
<tr>
<td>KPS-1®</td>
<td>25</td>
<td>80</td>
<td>Phosphate HEPES (7.4)</td>
<td>+</td>
<td>5</td>
<td>+</td>
<td>HES (50 g/l)</td>
</tr>
<tr>
<td>Lifor™</td>
<td>16</td>
<td>98</td>
<td>Most commonly HEPES (7.1)</td>
<td>+</td>
<td>0.01</td>
<td>+</td>
<td>Dextran 70 and/or HES and/or (45–55 g/l)</td>
</tr>
<tr>
<td>Celsior®</td>
<td>15</td>
<td>100</td>
<td>Histidine (7.3)</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Polysol®</td>
<td>15</td>
<td>120</td>
<td>HEPES phosphate histidine (7.4)</td>
<td>+</td>
<td>5</td>
<td>+</td>
<td>PEG 35 kDa (20 g/l)</td>
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<tr>
<td>Custodiol® HTK</td>
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<td>15</td>
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<td>–</td>
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<td>Extracellular type solution [K⁺] &lt; 7 mM</td>
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<tr>
<td>SCOT15®</td>
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<td>118</td>
<td>Carbonate (7.4)</td>
<td>+</td>
<td>0</td>
<td>–</td>
<td>PEG 20 kDa (15 g/l)</td>
</tr>
</tbody>
</table>

Bon, D. et al. (2012) New strategies to optimize kidney recovery and preservation in transplantation

Human Kidney
Decreased injury biomarkers with optimized normothermic perfusion

**Urinary NGAL**

- PRBC
- WB

![Graph showing Urinary NGAL levels over time with error bars and asterisk indicating statistical significance at 0.0202.]

**Urinary KIM1**

- PRBC
- WB

![Graph showing Urinary KIM1 levels over time with error bars.]

**Statistical Significance:**
- NGAL: *p* = 0.0202
- KIM1: Not explicitly stated
Acknowledgement

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