Induction of tolerance to allogeneic transplants – from preclinical models to clinical translation

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Initiation of an anti-transplant immune response

- **Donor / Graft Dendritic Cells**: Present allogeneic MHC alleles to CD4+ T cells directly and indirectly.
- **Secondary Lymphoid Organs**: Presentation of MHC alleles to CD4+ T cells.
- **Activation and Recruitment of Other Effector Cells**: CTL, CD8+ T cells, and CD4+ T cells.
- **B Cells**: Help in the immune response.
- **DC Licensing**: Involvement of DC licensing in the immune response.
## Current standard immunosuppression

<table>
<thead>
<tr>
<th></th>
<th>Calcineurin inhibitors</th>
<th>mTOR inhibitors</th>
<th>Anti-metabolites (methotrexate, MMF...)</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>mode of action</td>
<td>inhibition of proximal T cell activation</td>
<td>inhibition of T cell differentiation and proliferation</td>
<td>inhibition of T and B cell proliferation</td>
<td>general inflammation inhibition</td>
</tr>
<tr>
<td>autoimmune diseases</td>
<td>no (rarely)</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>organ transplantation</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>application</td>
<td>permanent</td>
<td>permanent</td>
<td>permanent</td>
<td>acute</td>
</tr>
<tr>
<td>side effects</td>
<td>nephrotoxicity, hypertension, (infections, malignancies)</td>
<td>hyperlipidemia, impaired wound healing</td>
<td>bone marrow suppression, gastritis / diarrhea</td>
<td>Diabetes, hypertension, osteoporosis</td>
</tr>
</tbody>
</table>

- Good short-term results, no improvement of long-term function
- Severe side effects cause high morbidity and mortality

**Tolerance induction or monimization of immunosuppression**
Definition of transplant tolerance

“Lack of a destructive immune response towards the graft in the absence of immunosuppression but presence of general immune competence”
Strategies to induce transplant tolerance

- **Induction of chimerism**
  - Stem cells + solid organ
  - negative selection (thymus)

- **Costimulatory blockade**
  - e.g. CTLA4-Ig/anti-CD40L

- **Depletion of lymphocytes**
  - e.g. anti-CD52/anti-CD20

- **CD3 or co-receptor targeting**

- **Application of donor material**
  - e.g. apoptotic donor cells

- **CD4 modulation**

- **Spontaneous tolerance**

- **Regulatory cells**
  - CD4^+CD25^{high}CD127^{low} Tregs
  - tolerogenic macrophages
  - tolerogeneic DCs...

- **Stem cells**

- **B cells**

- **T cells**
  - CD4^+CD8^+

- **Donor-reactive**

- **Donor alleles**

- **Thymus**
Induction of a chimerism

1. Irradiation (e.g. TBI = total body irradiation) and / or chemotherapeutic conditioning (e.g. cyclophosphamide) to destroy recipients own hematopoietic cells
2. Donor bone marrow (BM) leads to reconstitution by donor immune cells e.g. DCs
3. Donor-reactive developing recipient T cells are deleted within the thymus by donor DCs leading to acceptance of donor skin grafts

Noga Or-Geva et al. Blood 2013;122:3238
Depletion / costimulatory blockade / CD4-targeting

1. Depletion of lymphocytes
e.g. anti-CD4/anti-CD8

2. Costimulatory blockade
e.g. CTLA4-Ig

3. CD4 targeting

1. long-term graft acceptance but, slight increase in fibrosis, proteinuria and glomerulosclerosis (=signs of chronic rejection) on day 150 post-transplant

2. long-term graft acceptance but, self-limiting acute rejection episode, development of chronic rejection

3. long-term graft acceptance with no fibrosis, proteinuria and glomerulosclerosis even one year post-transplant, induction of highly efficient donor-reactive Tregs

Transfer of regulatory T cells

**CD4^{+}CD25^{high}\text{F}oxp3^{+} \text{T cells}**

- CD69/CD40L
- IFN-\gamma
- Foxp3^{+} T conv

**10\times10^{6}/kg b.w.**
**CD4^{+}CD25^{+} allo-reactive Tregs**

**DA (RT.1^{av/})**
**rat orthotopic kidney transplantation**

**LEW (RT.1^{i})**

**Suppression / Infectious tolerance**

- Apoptose
- Granzyme
- Perforin
- TGF-\beta
- IL-2
- IL-10
- IL-35
- cAMP
- Adenosine
- Adenosine

**APC Modification**

- CTLA-4
- CD80
- CD86
- IDO
- CD93

**DC**

**DA (RT.1^{av/})**
**LEW (RT.1^{i})**

- No increase in fibrosis, proteinuria and glomerulosclerosis on day 150 post-transplant

Graph showing percent survival over time after transplantation:

- untreated
- adoptive transfer

**21.04.2016**
**Clinical translation**

- **Depletion of lymphocytes**
  - e.g. anti-CD52/anti-CD20
  - **Not successful**

- **CD3 or co-receptor targeting**
  - **Not tested yet**

- **Costimulatory blockade**
  - e.g. CTLA4-Ig/anti-CD40L
  - **Not successful**

- **Application of donor material**
  - e.g. apoptotic donor cells
  - **Not tested yet**

- **Induction of chimerism**
  - Stem cells + solid organ
  - Successful (50%)
  - (thymus)

- **Spontaneous tolerance**
  - Observed „operational tolerance“

- **Regulatory cells**
  - CD4+CD25^high^CD127^low^ Tregs
  - Ongoing e.g. „ONE Study“
  - tolerogenic DCs...
Clinical translation – induction of chimerism


- Immunosuppression withdrawal performed in 8 out of 10 patients
- Successful tolerance induction is associated with reduction in donor-reactive T cell clones
- Some patients now developed chronic (humoral) rejection
- 4 patients still of all Immunosuppression (more than 5 years after transplantation
Clinical translation – induction of chimerism

Leventhal et al. Sci Transl Med 2012

-4 -3 -2 -1 0 1 180 – 360 days

200cGy total body

Fludarabine + Cyclophosphamide

Tacrolimus + MMF

several HLA MM

HSC enriched for tolerance promoting facilitating cells (e.g. p-preDCs)

Long lasting donor chimerism in several patients

IS withdrawal successful in 12 out of 19 patients

Long lasting T cell depletion especially CD4⁺ T cells

Graft loss (2 out of 19) due to infectious complications (viral and bacterial sepsis)
Clinical translation

**Depletion of lymphocytes**
e.g. anti-CD52/anti-CD20

**Not successful**

**CD3 or co-receptor targeting**

**Not tested yet**

**Induction of chimerism**
Stem cells + solid organ

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**Not successful**

**Application of donor material**
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**Not tested yet**

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**Observed „operational tolerance“**

**Regulatory cells**
- CD4+CD25<sub>high</sub>CD127<sub>low</sub> Tregs
- Ongoing e.g. „ONE Study“
- tolerogenic DCs...
Clinical translation – costimulatory blockade

BENEFIT = Phase III

Belatacept (mutated CTL4-4Ig) versus CsA
+ Basiliximab (anti-CD25 mAb) + Steroids + MMF

Vincenti et al. Am J Transpl 2012

<table>
<thead>
<tr>
<th>Arm 1 = More intense Belatacept n = 219</th>
<th>36 months</th>
<th>AR</th>
<th>PTLD</th>
<th>GFR</th>
<th>DSA</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>24%</td>
<td>3</td>
<td>65.2</td>
<td>6%</td>
<td>92%</td>
</tr>
</tbody>
</table>

| Arm 2 = Less intense Belatacept n = 226 |          | 17% | 2    | 65.8 | 5%   | 92%      |

| Arm 3 = CsA n = 221                     |          | 10% | 1    | 44.4 | 11%  | 89%      |

No cessation / weaning of immunosuppression possible!
Higher incidence of AR caused by costimulation-independent memory T cells?
Hurdles for tolerance induction

- underlying diseases (e.g. autoimmune hepatitis, FSGS, type-1-diabetes)
- treatment-resistant pre-formed donor-reactive or „cross“-reactive memory T and B cells

Kidney Tx patients on belatacept (CTLA4-Ig)

Espinosa Am J Transpl 2015

IL17 producing T effector cells are regulated by CTLA4- and PD-L1 engagement

Krummey J Immunol 2014,
Schumann Plos ONE 2015
Clinical translation

Depletion of lymphocytes
e.g. anti-CD52/anti-CD20

Not successful

CD3 or co-receptor targeting

Not tested yet

Stem cells + solid organ

Induction of chimerism

Successful (50%)

Stem cells + solid organ

Costimulatory blockade
e.g. CTLA4-Ig/anti-CD40L

Not successful

Application of donor material
e.g. apoptotic donor cells

Not tested yet

Spontaneous tolerance

Not tested yet

T cells
CD4+
CD8+
donor-reactive

CD4 modulation

B cells
donor-reactive

CD4+CD25^{high}CD127^{low} Tregs

Regulatory cells

Ongoing e.g. „ONE Study“

tolerogenic DCs...

Observed „operational tolerance“
Clinical translation – Transfer of regulatory T cells

A Clinical Trial of Regulatory T Cell-Based Immunotherapy for Tolerance Induction in Living Donor Liver Transplantation

Todo et al. Hepatology 2016
Clinical translation – Transfer of regulatory T cells

<table>
<thead>
<tr>
<th>Case</th>
<th>POD</th>
<th>Drug-free (month)</th>
<th>Liver function (U/ml)</th>
<th>LDLT</th>
<th>DSA(MFI)</th>
<th>SI (Anti-donor/anti-third)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,620</td>
<td>Off (33 mo)</td>
<td>AST/ALT/r-GTP</td>
<td>negative</td>
<td>negative</td>
<td>ND/ND</td>
</tr>
<tr>
<td>2</td>
<td>1,543</td>
<td>Off (31 mo)</td>
<td>26/26/14</td>
<td>negative</td>
<td>C-II.DQ7(10117)</td>
<td>9.2/8.6*</td>
</tr>
<tr>
<td>3</td>
<td>1,515</td>
<td>Off (32 mo)</td>
<td>23/21/79</td>
<td>negative</td>
<td>C-II.DQ7(5655)</td>
<td>2.9/2.9</td>
</tr>
<tr>
<td>4</td>
<td>1,410</td>
<td>Off (29 mo)</td>
<td>23/4/10</td>
<td>negative</td>
<td>C-II.DQ7(7042)</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>1,326</td>
<td>Tac (4 mg, x1/d)</td>
<td>17/12/25</td>
<td>negative</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>1,284</td>
<td>MMF (500 mg/d)</td>
<td>27/21/25</td>
<td>C-I.CW6(7099)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>1,263</td>
<td>Off (23 mo)</td>
<td>33/39/24</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>8</td>
<td>1,186</td>
<td>Off (18 mo)</td>
<td>18/13/20</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
</tr>
<tr>
<td>9</td>
<td>1,123</td>
<td>Tac (4 mg, x1/d)</td>
<td>20/13/18</td>
<td>C-II.DR1(5583)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>1,018</td>
<td>Off (16 mo)</td>
<td>18/13/16</td>
<td>negative</td>
<td>negative</td>
<td>ND</td>
</tr>
</tbody>
</table>

POD: postoperative days. AST: aspartate aminotransferase. ALT: alanine aminotransferase. r-GTP: gamma-glutamyl transpeptidase.


Todo et al. Hepatology 2016

• failure of tolerance induction, patients suffered from autoimmune disease (PBC/PSC)

Fantastic results, BUT heterogeneous not well characterized cell product, no controlled study (reference arm w/o cells ethical?), no dose escalation, no immune monitoring!
Clinical translation – Transfer of regulatory T cells

EU-funded (FP7) project to test safety and partially efficacy of regulatory cell therapy in kidney transplantation

Haematopoietic Regulatory Cells

Kidney Tx Recipients (living donation)
Production of regulatory T cells

1. Polyclonal Treg Expansion
(Berlin, London, Oxford)

2. Enrichment of donor-reactive Tregs
(San Francisco)

Donor “cells” (e.g. B cells) +

- RECIPIENT Sorted Tregs (CD4⁺CD25\text{high}CD127\text{low})
- donor-reactive Tregs

expansion with CD3/CD28 expansion beads)

Expanded donor-reactive Tregs
ONE Study clinical trial design

"Primary Endpoint" (rejection-BPAR)

Reference Group Trial (n>60 total)
enrollment finished, follow-up nearly finished
AR: 15%

Cell Therapy Trial (n=8-16 per center)
>20 patients treated (10x Oxford + London poly Tregs, 1x Regensburg Mregs, 8 Berlin poly Tregs, 2 Boston, 1 San Francisco...)
AR: 5%

cell dose escalation
e.g. nTregs in Oxford:
1x10E6, 3x10E6, 6x10E6, 10x10E6/b.w.
### Immune monitoring within the ONE Study

<table>
<thead>
<tr>
<th>IM ASSAY</th>
<th>Safety</th>
<th>Efficacy</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Viral Load</td>
<td>A. CMV, EBV, BKV</td>
<td>C. Gene expression of operational tolerance versus rejection</td>
<td>B. Signs of Immunodeperession or paralysis</td>
</tr>
<tr>
<td>C. Gene Expression</td>
<td>C. Gene expression of operational tolerance versus rejection</td>
<td>D. subsets defining operational tolerance versus rejection</td>
<td>C. Immediate change in leukocyte subsets upon cell transfer</td>
</tr>
<tr>
<td>D. Leucocyte Profiling</td>
<td>E. occurrence of anti-donor HLA antibodies</td>
<td>G. Frequence of e.g. IFNg producing allo-reactive memory T cells</td>
<td>D. Immediate change in gene expression</td>
</tr>
<tr>
<td>E. Anti-donor Abs</td>
<td>F. Frequence of e.g. IFNg producing allo-reactive memory T cells</td>
<td>G. Frequence of antigen-reactive effector and regulatory T cells</td>
<td></td>
</tr>
<tr>
<td>F. T cells (ELISPOT)</td>
<td>H. Stability (TSDR demethylation) of Tregs</td>
<td>H. Stability (TSDR demethylation) of Tregs</td>
<td></td>
</tr>
<tr>
<td>G. T cells (CD154/137)</td>
<td>I. Chemokines as sign of ischemia or rejection</td>
<td>I. Chemokines as sign of ischemia or rejection</td>
<td></td>
</tr>
<tr>
<td>H. Tregs (FOXP3)</td>
<td>J. Donor-specific regulation</td>
<td>J. Donor-specific regulation</td>
<td></td>
</tr>
<tr>
<td>I. Urinary IP-10</td>
<td></td>
<td></td>
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<tr>
<td>J. Immune Regulation</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>B. HLA-DR Levels</td>
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<tr>
<td>D. Leucocyte Profiling</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>K. Microarray</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Trzonkowski et al. Sci Transl Med 2015
Summary / conclusions

- Active transplant tolerance induction is possible (e.g. chimerism, Tregs)
- Other approaches are or should be tested in selected patient cohorts
- Better understanding of immune mechanisms influencing outcome in individual patients is needed
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