The mechanisms of accommodation in ABO-incompatible kidney transplantation -from bed side to bench-

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The authors have no financial conflicts of interest to disclose concerning the presentation.
Case 1. 33y.o Male ABO incompatible KTx (B→O)

Serum Creatinine

DFPP  Splenectomy  PEX  Graftectomy

<table>
<thead>
<tr>
<th>Days</th>
<th>TUC</th>
<th>Trough 10-30ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre</td>
<td></td>
<td>125</td>
</tr>
<tr>
<td>-14</td>
<td>125</td>
<td>100</td>
</tr>
<tr>
<td>-13</td>
<td>100</td>
<td>80</td>
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<tr>
<td>-12</td>
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<td>-11</td>
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<td>-9</td>
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<tr>
<td>-8</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>-7</td>
<td>500</td>
<td>250</td>
</tr>
<tr>
<td>-6</td>
<td>250</td>
<td>125</td>
</tr>
</tbody>
</table>

Anti-B Ab IgG

| Days | 16 | 8 | 4 | 2 | <2 | <2 | 2 | 4 | 32 | 64 |

Anti-B Ab IgM

| Days | 4 | 4 | 2 | 2 | <1 | <1 | 8 | 64 | 256 | 256 |
Immunological reaction in ABO-incompatible renal grafts

Mina Hur, et al. Understanding the Complexities of Kidney Transplantation
Excellent Long-term Outcome of ABO-Incompatible Living Donor Kidney Transplantation in Japan

- ABO-incompatible KTx (n=564)
- ABO-compatible KTx (n=1055)

P value: not significant

(Kaplan-Meier)
Accommodation
Accommodation refers to the phenomenon in which rejection does not clinically occur despite the presence of antigens on graft vascular endothelium and the presence of antibodies in the blood of recipients.
The induction of accommodation in ABOi KTx

Antibody reaction to A/B antigens induce inhibition of ERK pathway and upregulation of CD55/CD59 which are complement inhibitors.
Case2. 23y.o male ABO incompatible KTx (B→O)

Cr (mg/dl)

MP 8mg 100 80 125 60 → 4
MMF 750mg 1500mg
FK Trough 2-4 ng/ml 10-12 ng/ml → 5-10 ng/ml

Rituximab 100mg
DFPP

Anti-B IgG 32 16 8 4 <2 <2 <2 <2 <2 <2 <2
Anti-B Ab IgM 64 32 16 8 4 2 1 1 1 1
Patients who underwent ABOi LKTx in Niigata Univ. between 1994.2-2014.5 (n=82)

Patients whose grafts survived and serum antibody titers could be serially recorded in Niigata Univ. (n=50)

Exclusion
- Anti-donor blood type Ab could not be recorded (Early era) (n=5)
- Follow in the associate institution (n=15)

Lost graft function (n=12)

Patients whose anti-donor blood type Ab ≤1:4 (Both IgG and IgM). (n=42)

Patients whose anti-donor blood type Ab ≥1:8 (Either IgG or IgM). (n=8)

In Vitro experiment for the patients that the informed consents were obtained. (n=30)


84%
The change of serum antibody titer after A-incompatible kidney transplantation

- **a. Anti-A IgG Ab (A1→O)**
- **b. Anti-A IgM Ab (A1→O)**
- **c. Anti-B IgG Ab (A1→O)**
- **d. Anti-B IgM Ab (A1→O)**

Anti-A antibody was persistently low after A-incompatible kidney transplantation.
The change of serum antibody titer after B-incompatible kidney transplantation

Anti-B antibody was persistently low after B-incompatible kidney transplantation
The change of serum antibody titer after ABO compatible kidney transplantation

Antibody titer were remained unchanged before and after ABO compatible kidney transplantation.
Serum antibody titer were specifically and continuously low level to donor blood group antigen after ABO incompatible kidney transplantation. Immunosuppression was not involved in the maintenance of this phenomenon by itself.
Antibody titers against donor blood type were *specifically* and *persistently* low after ABOi KTx.

- Inhibition of antibody production?
- Antibody absorption by the graft?
Antibody production function analysis *in-vitro* experiment

**Isolation PBMC**

**Cultured for 6 days**
- Medium: AIM-V
- Stimulator: CpG, IL10, IL15, IL2
- Day4: change medium
- Day7: Take and save the sup
  - Harvest cultured cells

**Analyze anti-A and B antibody in supernatant by ELISA**
Anti-A antibody production was low in patients who received A-incompatible renal grafts.
Anti-B antibody production was low in patients who received B-incompatible renal grafts. Donor specific antibody production function was down regulated in ABO-incompatible KTx. (NOT by absorption)
Acquired Downregulation of Donor-Specific Antibody Production After ABO-Incompatible Kidney Transplantation

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FITC, fluorescein isothiocyanate; LKTx, living-donor kidney transplantation; PBMC, peripheral mononuclear cell; PE, phycoerythrin; PerCP, peridinin-chlorophyll-protein; PTC, peritubular capillary; RBCs, red blood cells; rh, recombinant human

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Control !!

Who !?
What is the mechanisms of down regulation?

✔️ Imunosuppression?

❌ The existence of ABO-incompatible kidney graft?
Antibody against self blood type is not produced after ABO minor mismatched bone marrow transplantation.

Anti-A Ab is not produced because A antigen is existed.
Case presentation

57y.o male received an ABOi KTx (B⇒O).

Anti-B Ab titer; IgGx32, IgMx32 before KTx.
  maintained IgG<1, IgMx1 after KTx

He stopped to take immunosuppressive medicines at all 6 years after KTx.
One year and a half have been past.
Serum creatinine level increased from 0.8 to 13.42 mg/dl and hemodialysis was started.

Anti-B Ab titer; remained IgG<1, IgMx1
Who else down-regulated the antibody production function against donor blood type antigen after ABO-incompatible kidney transplantation?

✓ Immunosuppression?
✓ The existence of ABO-incompatible kidney graft?
✓ Regulatory B cell?
Who else down-regulated the antibody production function against donor blood type antigen after ABO-incompatible kidney transplantation?

Objective:
The analysis of B cell population in peripheral blood.

Materials and methods
Blood samples were obtained healthy volunteers (n=20), ABO-compatible KTx recipients (n=20), and ABO-incompatible KTx recipients (n=20). B cell population was analyzed by flow cytometry.
IL-10 producing B cells

- IL-10
- Iso type

CD19+IL-10+/CD19+

- (%)
- Healthy volunteers
- ABO incompatible
- ABO compatible
Transitional B cell

CD24hiCD38hi/CD19+

<table>
<thead>
<tr>
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<th>(%)</th>
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<tbody>
<tr>
<td>Healthy volunteers</td>
<td>8</td>
</tr>
<tr>
<td>ABO incompatible</td>
<td>6</td>
</tr>
<tr>
<td>ABO compatible</td>
<td>4</td>
</tr>
</tbody>
</table>

P = 0.01

P = 0.06
The rate of CD5 positive B cells were high in ABO-incompatible kidney transplant recipients.
Multiple regulatory mechanisms control B-1 B cell activation

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**FIGURE 1** Regulation of B-1 B cell activation. (1 and 2) CD5 mediated regulation – CD5 acts as an anchor for SHP-1 recruitment on cell surface near BCR signaling complex, which in turn inhibits BCR signaling. (3) CD19 mediated regulation – B-1 cells have defective Vav recruitment to CD19 leading to reduced Ca\textsuperscript{2+} mobilization and cell activation upon BCR co-stimulation. (4) Src family kinase mediated regulation – Src family kinase, Lyn, plays an essential role in phosphorylation of CD5 and subsequent recruitment of SHP-1 on CD5. (5) IL-10 mediated regulation – B-1 cells make high levels of IL-10 upon TLR and BCR stimulation, which work in an autocrine manner and inhibit B-1 cell responses by blocking degradation of IкB\textalpha{} and RelA translocation to the nucleus.
Accommodation in ABO incompatible KTx

CD5+ B cell

Donor ABO antigens

Anergy
Case 3. 33y.o Male, **Very high** Ab titer of B→O KTx

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg)</th>
<th>Schedule</th>
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<tbody>
<tr>
<td>CYA po</td>
<td>250</td>
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</tr>
<tr>
<td>iv2mg/kg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>po300</td>
<td>350</td>
<td>300</td>
</tr>
<tr>
<td>MMF 2000</td>
<td>2500</td>
<td>1500</td>
</tr>
<tr>
<td>MP 8mg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rituximab 100mg</td>
<td></td>
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<tr>
<td>Rituximab 100mg</td>
<td></td>
<td>100mg</td>
</tr>
<tr>
<td>DFPP 100mg</td>
<td>500mg<del>250</del></td>
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<tr>
<td>Basiliximab 20mg</td>
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<tr>
<td>Basiliximab 20mg</td>
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<td>20mg</td>
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**SCr (mg/dl)**

- **Days**
  - 28
  - 14
  - 8
  - 6
  - 4
  - 2
  - 0
  - 2
  - 4
  - 6
  - 8
  - 10
  - 12
  - 14
  - 16

**S-Cr**

- 2048 x
- 256 x
- 16 x

**Anti-B IgG**

- 2048 x
- 256 x
- 16 x

**Dose this antibody react to kidney?**
Antibody titer is measured by agglutinin assay using red blood cells.
The differences of the proteins linked to ABO antigens between kidney and RBC

- ABO antigens on endothelium
- Homogenate
- Lectin column chromatography
- Proteomic analysis
  - PECAM1
  - PLVAP
  - vWf

SDS-PAGE/Westernblot

PECAM1 represented the major 130-kDa type ABO antigen-carrying protein in the human kidney.
Identification and Characterization of Major Proteins Carrying ABO Blood Group Antigens in the Human Kidney

Masayuki Tasaki,1,2 Yutaka Yoshida,1,5 Masahito Miyamoto,1,3 Masaaki Nameta,4 Lino M. Cuellar,1 Bo Xu,1 Ying Zhang,1 Eishin Yaoita,1 Yuki Nakagawa,2 Kazuhide Saito,2 Tadashi Yamamoto,1 and Kota Takahashi2

(Tasaki M et al. Transplantation 2009;87: 1125–1133)
Antibody reacts to red blood cells and kidney, but the reaction is NOT equal
Summary

- Donor-specific downregulation of the capability to produce antibodies was acquired in ABO-incompatible KTx.
- ABO-incompatible graft and CD5 positive B cells may contribute to establish accommodation.
Summary

- The reaction against red blood cells and kidney may NOT be equal.
- The method to measure antibody titer which react to ABO antigens on the endothelial cells should be developed.
Thank you for your attention.