B cells in transplantation

Dr Menna R Clatworthy
University Lecturer in Transplantation Medicine

Paris, April 26th 2016
Outline

1. B CELLS IN TRANSPLANTATION
2. SOME BASIC B CELL BIOLOGY
3. FROM BENCH BASICS TO BEDSIDE
4. FUTURE CONCEPTS FOR B CELL TARGETING
I. B cells in transplantation
Immunotherapy

- Belatacept
- MMF
- SIR
- Anti-CD25
- Tacrolimus
- Ciclosporin
- Pred/AZA

TCMR

- 1970 to 1980: 70%
- 1990: 50%
- 2000: 20-30%
- 2010: 10-20%
Alloantibodies in transplantation

Antigen binding
(HLA/non-HLA)

B cells produce antibodies

B cells in transplantation

Non-sensitised patient

Naïve B cell → Plasma cell

De novo DSA - 80% heart Tx
25-30% kidney/lung Tx

Smith et al. Am J Transplant 2011;11:312-319
Antibodies as immune effectors

Complement

Fcγ Receptor

Pathogen neutralisation

Complement C1q

MAC

Antibodies as immune effectors
Antibodies as immune effectors
B cells in rejection

Non-sensitised patient

Naïve B cell  →  Plasma cell  →  Endothelial cells

De novo DSA – 80% heart Tx
25-30% kidney/lung Tx

Smith et al. Am J Transplant 2011;11:312-319

αABMR 5%
Acute antibody-mediated rejection

Endothelialitis

CD4d+
B cells in rejection

Non-sensitised patient

Naïve B cell → Plasma cell

Sensitised patient

Memory B cell → Plasma cell

30% sensitised > ABMR 50%

Loupy et al. N Engl J Med 2013;369:1215-1226-
DSA associated with chronic allograft damage

Chronic antibody-mediated rejection

Fibrointimal hyperplasia

Glomerulopathy

IFTA
DSA associated with allograft loss

- **Probability of Graft Survival**
  - Years after Transplantation
  - DSA- vs. DSA+

- **No. at Risk**
  - DSA-: 700, 698, 667, 612, 504, 338, 164, 38
  - DSA+: 316, 312, 295, 229, 176, 100, 56, 19

- **P < 0.001 by log-rank test**
The Treatment of Acute Antibody-Mediated Rejection in Kidney Transplant Recipients—A Systematic Review

Darren M. Roberts, Simon H. Jiang, and Steven J. Chadban

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The Treatment of Acute Antibody-Mediated Rejection in Kidney Transplant Recipients—A Systematic Review

Darren M. Roberts,1,2,4 Simon H. Jiang,1 and Steven J. Chadban1,3

Controlled Trials
N=12

Randomised
N=5

Non-randomised
N=7

Median patients/arm =13 (5-23)

PEX
N=4

IA
N=1
Rituximab in ABMR

- RITUX-ERAH. Phase III, double-blind, placebo-controlled trial
- N=38 with ABMR - rituximab (375 mg/m) or placebo at day 5
- All patients received PE, IVlg, and CS
- Primary EP: graft loss or no improvement in GFR at D12

- **RESULTS**
  - Primary EP: Rituximab = 52.6% (10/19) Placebo = 57.9% (11/19)
  - No difference in serum creatinine level and proteinuria at 1, 3, 6, and 12 months
  - Supplementary administration of rituximab to 8/19 patients in placebo group

Summary 1: B cells in transplantation

Antibody producers


ABMR

Antigen presenters


TCMR

An unmet need
2. B cell biology basics
B cell activation

B cell → Plasma cell → Antibody
B cell activation

Clatworthy MR. Am J Transplant 2011
Co-activating receptors

Inhibitory receptors

Activating signalling

PI3K

PPI2

PIP3

BCR

Igα Igβ

ITAM

Activation threshold

Activation threshold
Co-activating receptors

CD19

CD21 (CR2)

BAFF-R

Inhibitory receptors

CD22

FcγRIIB

Activating signalling

Activation threshold
BAFF and APRIL

- B cell survival
- Plasma cell survival
- T-dependent antibody response

• Class switching
• T-independent (2) antibody responses
B cell activation

**B cell activation**

- Naïve B cell
- GC B cell
- Tfh
- PB
- PC
- Memory B cell
- IgG
- IgM
- BAFF
- IL21
- CO-STIMULATION
- FcγRIIB

**Cell Types:**

- **Naïve B cell**
- **GC B cell**
- **Tfh**
- **PC**
- **Memory B cell**

**Antibodies:**

- **IgM**
- **IgG**
B cell activation

IL-21 and CD40L Synergistically Promote Plasma Cell Differentiation through Upregulation of Blimp-1 in Human B Cells

B. Belinda Ding,1 Enguang Bi,1 Hongshan Chen, J. Jessica Yu, and B. Hilda Ye

J Immunol 2013;190:1827-36
Plasma cell adaptation for longevity

Inactivation leads to loss of BM plasma cells

**prdm1**

BLIMP1

XBP1

Control of ER stress

B cell activation

Naïve B cell

GC B cell

CO-STIMULATION

Tfh

PB

PC

IgM

FcγRIIB

BAFF

IL21

IL6

APRIL

BAFF

Eo

Nφ

Mφ

IgG

Memory B cell
Eosinophils are required for the maintenance of plasma cells in the bone marrow

Van Trung Chu¹, Anja Fröhlich¹, ², Gudrun Steinhauser¹, Tobias Scheel¹, Toralf Roch¹, ⁴, Simon Fillatreau¹, James J Lee³, Max Löhning¹, ² & Claudia Berek¹

Eosinophils co-localise with plasma cells
Eosinophils deficient mice have reduced APRIL and IL6 in BM
3. From bench basics to bedside
B cell targeting in Tx

Naïve B cell

PC

GC B cell

Tfh

IgM

BAFF

IL21

FcyRIIB

Anti-BAFF

IgG

Memory B cell

PB

Eo

Nφ

Mφ

CO-STIMULATION

IL6

BAFF

APRIL
BAFF inhibition improves allograft survival

- **Murine cardiac allograft model:**
  - BAFF-/- recipients had extended Tx survival (Ye et al. EJI 2004; 34(10):2750-9)
  - Deleterious effect of BAFF dependent on BAFF-R, and independent of TACI and BCMA

- **Murine islet allograft model:**
  - Monoclonal a-BAFF antibody (+rapamycin at induction) -> long-term survival of MHC-disparate allografts (Parsons et al. Transplant 2012; 93(7):676-85).
Serum BAFF as a biomarker

- N=143 patients
- Stable graft function
- sBAFF, BAFFmRNA, BAFF-R assessed
- Long-term follow-up

- High serum BAFF = 5x in risk of DSA

Serum BAFF as a biomarker

**BAFF levels in antibody-incompatible transplants (n=32)**

Phase 2 trial of BAFF blockade in kidney transplantation

- Mechanistic and safety study of belimumab (BEL114424)
- N=20 low risk renal transplant recipients

Belimumab (10mg/kg) or Placebo

Induction immunosuppression (Basiliximab, Methylprednisolone)

Maintenance immunosuppression (FK, MMF, Prednisolone)

Time (months)

0 1 2 3 4 5 6 7 8 9 10 11 12

Study visit and venepuncture
B cell targeting in Tx

**Naïve B cell**
- IgM
- FcγRIIB

**Memory B cell**
- IgG

**GC B cell**
- CO-STIMULATION
- IL21

**Tfh**
- BAFF
- APRIL
- IL6

**PC**
- Eo
- Nφ

**Co-stimulatory blockade**
- IL21-IL21R blockade

**Co-stimulatory blockade**
Belatacept

Fusion protein
(CTLA-4 extracellular domain fused to the Fc portion of IgG1)
Induction: Basiliximab

Maintenance: Prednisolone, MMF

1 : 1 : 1

BELA (MI)

BELA (LI)

CsA

7 year follow-up data

The cumulative event rates of DN DSA at Years 3, 5, and 7:

bela MI = 2.32, 6.21, and 6.21
bela LI = 1.52, 2.39, and 4.48.
CsA = 11.25, 17.07, and 21.30

Vincenti et al. NEJM 2016
B cell targeting in Tx

Co-stimulatory blockade
IL21-IL21R blockade
Tfh and IL21 in transplantation

- Tfh increased in patients with pre-formed DSA
- Memory B cells $\rightarrow$ Plasmablast inhibited by IL21R

B cell targeting in Tx

- Proteasome inhibitors
- BAFF/APRIL blockade
- IL6R (Tocilizumab)
- Eosinophil depletion
Proteasome inhibitors

Misfolded protein

BORTEZOMIB

26S Proteasome

Misfolded protein

Misfolded protein

Misfolded protein

APOPTOSIS
Proteasome inhibitors

BORTEZOMIB

Plasma cell

Licensed for use in multiple myeloma

APOPTOSIS
Bortezomib reduces DSA

- Murine cardiac transplantation model

Bortezomib in ABMR

- Historical control study in refractory ABMR
- 10 bortezomib-treated patients (4 x 1.3 mg/m²)
- 9 rituximab-treated patients (1 x 500 mg)
- 18-month graft survival 60% vs. 11% in bortezomib v rituximab group
- Adverse events:
  - Diarrhoea, Nausea/vomiting, Neuropathy


- BORTEJECT - single-center study, 44 subjects with biopsy-proven ABMR to a double-blind placebo-controlled intervention trial.

Second generation proteasome inhibitors

Carfilzomib
• Phase II study in treatment of ABMR in lung transplantation (ClinicalTrials.gov Identifier: NCT02474927)
• Sensitised wait-listed kidney transplant recipients -> efficacy in reducing HLA antibodies (ClinicalTrials.gov Identifier: NCT02442648).

Ixazomib
• Orally administered proteasome inhibitor
• Used in combination with low dose ciclosporin in rat model of kidney transplantation.
• Ixazomib reduced DSA in both sensitised animals and in those with de novo post-transplant DSA.

B cell targeting in Tx

- Naïve B cell
- GC B cell
- Tfh
- PB
- PC
- Memory B cell
- IgG

• Proteasome inhibitors
• BAFF/APRIL blockade
• IL6R (Tocilizumab)
• Eosinophil depletion
Targeting the niche – eosinophils depletion

- Siglec-F antibody ineffective in mice

- IL5 blockade depletes eosinophils (in mice/humans) & reduces BM PCs in skin allograft model

Targeting the niche – eosinophil depletion

B cell targeting in Tx

- Naïve B cell
  - IgM
  - FcγRIIB
- GC B cell
  - CO-STIMULATION
  - IL21
- Tfh
- PC
- IgG
- Memory B cell
  - Memory B cell
- Eosinophil
- Nφ
- Mφ

**Anti-BAFF**

**Co-stimulatory blockade**

**IL21-IL21R blockade**

- Proteasome inhibitors
- BAFF/APRIL blockade
- IL6R (Tocilizumab)
- Eosinophil depletion
Bashing B cells not always good

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<td>Rejection at 6 months</td>
<td>12 v 18%</td>
<td>83 v 14%</td>
<td>17 v 21%</td>
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B cell depletion associated with TCMR

RCT
Renal transplants
N=120

**Daclizumab**
(D0+D7 1mg/kg)

**Rituximab**
(D0+D7 10mg/kg)

**Tacrolimus**
(0.075mg/kg, 8-15ng/ml)

**MMF**
(1g bd)

B cell depletion associated with 83% TCMR cf 14% in the control group

*Clatworthy et al. NEJM 2009;360(25):2683-5*

...and rejection in some murine models.

*DiLillo et al. J Immunol 2011;186(4):2643-54*
EFFECTORS

REGULATORS
Regulatory B cells/ B10 cells

**Mice**
- CD1d
- CD2
- TIM-1
- IL10

**Humans**
- CD24
- CD27
- CD38
- CD71
- CD25

**Regulators**
- TIM-1
- IL10

References:
- Blair PA et al. *Immunity*. 2010; 32: 129-40
Regulatory B cells in transplantation

• Tolerant subjects – B cell signature in transcriptomic analysis of PBMCs. Newell et al. J Clin Invest 2010
Regulatory B cells in transplantation


- Increased IL10 production in peripheral B cells. Chesneau et al. Am J Transplant 2014
Regulatory B cells in transplantation

- Increased IL10 production in peripheral B cells. Chesneau et al. Am J Transplant 2014
4. Future concepts for therapeutic targeting
Patient with ESRF

Immunosuppression
Patient with ESRF

Immunosuppression
Understanding recipient risk

DNA variation

mRNA

Protein

Genetic studies

Transcriptomics

Biomarkers
Understanding recipient risk

B cell → Plasma cell → ABMR

Protein Biomarkers

☑️ ☑️
HLA antibodies and risk stratification

**Memory B cell**

**GC B cell**

**Naïve B cell**

**Tfh**

**IgG**

**IgM**

**FCγRIIB**

**FCγRIIB**

**BaFF**

**IL21**

**CO-STIMULATION**

**PB**

**PC**

**Eo**

**Nφ**

**Mφ**

**Co-stimulation**

**De novo DSA+ +/- acute ABMR**

**Sensitised, DSA+**

**HLA-**
### Understanding recipient risk

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<th>GENOTYPE/BIOMARKER</th>
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<td>IL10 -1082 (A) -819 (C) -592 (C) haplotype</td>
<td>Allograft loss</td>
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<td>gene studies</td>
<td>TGF-β +869 (C), +915 (C) haplotype</td>
<td>5yr graft survival / function</td>
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<td>mRNA Transcipton</td>
<td>CD20 mRNA (urine)</td>
<td>Tolerance</td>
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<td>Multiple B cells transcripts (PBMC)</td>
<td>Rejection-free survival</td>
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<td>MS4A1 (CD20), TCL1A, and CD79B (PBMC)</td>
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<td>CD20, CD74, Ig heavy / light chains (kidney)</td>
<td>‘molecular Dx’ TCMR</td>
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<td>CD72 and BTLA (Kidney)</td>
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Take home messages

1. Antibodies cause significant graft pathology
2. Targeting B /plasma cells is an unmet need
3. Basic science-Experimental medicine partnership critical
4. Spare the regulators?
5. Stratification by genotype, phenotype, biomarker required to optimise application of B cell targeting
• Rituximab trial – Chris Watson, Vicky Bardsley, Afzal Chaudhry, Andrew Bradley, Ken Smith

• Serum BAFF – Gemma Banham, Davide Prezzi, Sarah Harford, Craig Taylor, Rizwan Hamar, Rob Higgins.