Fellows Symposium on Transplantation Medicine

Sunday, September 25
8:30 am - 9:05 am

Biomarkers and Surrogate Markers in Transplantation

Peter S. Heeger, MD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org
Biomarkers to predict outcomes in Transplantation: Is it possible?

Peter S. Heeger, MD
Professor of Medicine
Mount Sinai School of Medicine
New York, NY

Definitions

- **Surrogate Endpoints** are findings or measurements used (in clinical trials) to evaluate the safety or effectiveness of a therapy and serve as alternatives to traditional endpoints.
- **Biomarkers** are anatomic, physiologic, biochemical, or molecular parameters that indicate, or are associated with an alteration in physiology and are of clinical significance (this doesn’t necessarily mean they are clinically useful).
- **Surrogate Markers** can be defined as biomarkers that have established clinical utility.

Endpoints in transplantation: What we are trying to predict?

- Patient death
- Graft failure
- Acute rejection
- Change in kidney function
- Chronic injury
- Tolerance/Stable function (to guide drug withdrawal)
- Over- or under-immunosuppression
- I/R injury; delayed graft function
- other

Ideal Biomarkers

- High positive and negative predictive value
- Sensitive—e.g. diagnoses rejection (acute and/or chronic) before its occurrence
- Rapid
- Noninvasive
- Easy to perform reliably
- Inexpensive

Battery rather than a single test!

Biomarker Development

- Discovery
  - Validation sets
- Prospective multicenter validation
  - Can the assay be used in “real” setting?
- Assay Standardization
- Commercialization
  - Reimbursement
  - Distribution
Biomarker Discovery

- Biased
  - Looking for known molecule or readout
  - Commonly developed following a basic science discovery addressing an underlying mechanism
- Unbiased
  - Genomic, proteomic, other screening approaches
  - No specific hypothesis going in except that it will be possible to detect differences among groups with distinct clinical phenotypes

Examples of Biomarkers in Transplantation

Anti-HLA antibody detection techniques

Concept of FlowPRA™ and ability to detect donor HLA reactive antibody

HLA antigens
Latex beads
Mixture of 30 beads

Anti-HLA antibodies portend poorer outcomes

- Anti HLA ab and DSA are causes of acute rejection and have been implicated as mediators of chronic injury (TGP, CAN)
- Approximately 20% of kidney transplant recipients have anti HLA antibodies (not necessarily DSA) but have good kidney function
- Graft failure occurs 2.7 (class I) to 4 (class II) years after the development of anti HLA antibodies
- 4 yr graft survival was 58% in patients with antibodies and 81% in patients without (158 patients)

All antibodies predict long term graft failure (Terasaki et al)

<table>
<thead>
<tr>
<th>Anti-HLA antibody testing ongoing issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercialization is complete but…</td>
</tr>
<tr>
<td>Limited by HLA alleles on beads</td>
</tr>
<tr>
<td>Not all alleles represented</td>
</tr>
<tr>
<td>Not all subtypes represented (sequence of HLA B7 on bead may be different from HLA B7 on donor)</td>
</tr>
<tr>
<td>Standardization</td>
</tr>
<tr>
<td>Reagents</td>
</tr>
<tr>
<td>Laboratory equipment</td>
</tr>
<tr>
<td>What is a positive?</td>
</tr>
</tbody>
</table>
Antibodies to non HLA antigens as biomarkers in transplantation

- Increasing clear that antibodies to non HLA antigens are detectable in organ transplant recipients
- Antibodies to self antigens have been associated with acute and chronic injury
- One example is anti-type V collagen and bronchiolitis obliterans following lung transplantation

Hypothesis driven biomarker development: Are antibodies to cardiac myosin (CM) associated with CAV in heart transplant recipients?

Cross-sectional Cohort from Mount Sinai
40 patients with chronic allograft injury as defined by angiographic evidence of Chronic Allograft Vasculopathy (CAV)
32 patients without angiographic evidence of CAV
Obtained serum and PBMC
Measured anti-CM antibodies and CM-reactive T cells
Correlated with presence or absence CAV in univariable and multivariable analyses

Kalache et al J Immunol 2011, in press

Anti-CM antibodies are more prevalent in patients with CAV

Anti-CM antibodies are more prevalent than other autoantibodies in patients with CAV

Kalache et al J Immunol 2011, in press

Anti-CM are more prevalent than anti-donor HLA antibodies in patients with CAV

Kalache et al J Immunol 2011, in press

Multivariable Logistic Regression of associations with CAV

Kalache et al J Immunol 2011, in press
Conclusion: anti-cardiac myosin immunity

- The results support the concept that autoimmunity to CM could be used as a biomarker for transplant vasculopathy and raise the possibility that this autoimmune response contributes to posttransplant injury.

Antibodies to non HLA antigens as biomarkers

- Still in the discovery phase
- Biomarker for chronic injury seems promising
- Prospective Validation ongoing
- Commercialization not yet initiated

Detection of donor reactive memory cells

- Among many characteristics, donor reactive memory cells are resistant to most immunosuppressants, are present at high frequency, have high functional avidity and respond rapidly to antigenic challenge
- Thus, while T cell memory protects against reinfection, memory is a detriment to transplantation

Hypothesis

Pretransplant frequency of anti donor effector/memory T cells as will predict posttransplant AR and or posttransplant renal function

Cytokine ELISPOT ASSAY

Pre-transplant donor-reactive T cells and post-transplant outcome (Univariable analysis)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative (&lt;25/300 K)</th>
<th>Positive (&gt; 25/300 K)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute cellular rejection</td>
<td>17%</td>
<td>90%</td>
<td>.036</td>
</tr>
<tr>
<td>GFR (MDRD) 12 months</td>
<td>55 ± 20 ml/min/1.73 m²</td>
<td>37 ± 16 ml/min/1.73 m²</td>
<td>.006</td>
</tr>
<tr>
<td>DGF</td>
<td>23%</td>
<td>31%</td>
<td>NS</td>
</tr>
</tbody>
</table>

Augustine et al, AJT 2005
Logistic regression: correlates with GFR at 12 mo

<table>
<thead>
<tr>
<th>Variable</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGF</td>
<td>0.010</td>
</tr>
<tr>
<td>AR (biopsy proven)</td>
<td>0.058</td>
</tr>
<tr>
<td>Pretransplant ELISPOTs</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Augustine et al JASN 2005 and unpublished

Validation sets

- Our initial findings have been verified independently using separate cohorts
- ELISPOTs pre and posttransplant correlate strongly with AR and late graft injury
  - Berlin group led by Volk, Reinke,
  - Korea, Madrid, Boston, Rotterdam, others

Prospective Multicenter Validation Clinical Trials in Organ Transplantation (CTOT) NIH U01, P Heeger PI

- Clinical Goal: Development of noninvasive tests to facilitate safe minimization of immunosuppression
- Funding Period: 2004-2009 NIAID

Pretransplant Donor-stimulated ELISPOTs for IFNγ (≥25 cells/300K) and Posttransplant eGFR

P value of t-test for difference in group means at time point

T cell memory as a biomarker for acute and chronic injury
Where do we stand?

- Discovery and multicenter validation from many sites suggest quantifying T cell memory reactive to donor is a promising biomarker for long term outcome
- Prospective testing of whether decision making guided by ELISPOT improves outcome is required
- Standardization ongoing
- Commercialization may be problematic
- Other technical approaches for measuring memory (e.g. flow cytometry) can provide similar data

Urinary chemokines as biomarkers for kidney transplant injury
Chemokines

Elevation of CXCR3-Binding Chemokines in Urine Indicates Acute Renal-Allograft Dysfunction

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Rejection</td>
<td>90.5%</td>
<td>84.2%</td>
<td>91.9%</td>
<td>89.2%</td>
</tr>
<tr>
<td>IF/TA</td>
<td>85.3%</td>
<td>91.4%</td>
<td>88.7%</td>
<td>90.2%</td>
</tr>
</tbody>
</table>

Urinary chemokines as biomarkers for kidney transplant outcomes

- Discovery and initial validation look promising for specific chemokines as biomarkers of acute rejection and possibly late IF/TA (Nickerson and colleagues, Transplantation 2010)
- Prospective validations in progress
- Commercialization
  - Potential for point of care diagnostics

CTOT09

Testing urinary chemokines as noninvasive biomarkers for rejection during CNI withdrawal

Cylex Immunknow

Repeatability of Urinary Chemokines

Cylex Immunknow

Urinary chemokines as noninvasive biomarkers for rejection during CNI withdrawal

- High levels trigger biopsy

Immunoknow

- Discovery and initial validation suggests relationship between test results and level of immunosuppression
- Standardization and Commercialization complete
- Need prospective multicenter studies to determine if making changes based on test results improves outcomes

Peter S. Heeger, MD
www.a-s-t.org
Urinary Gene expression profiling as biomarkers for acute rejection

CTOT04
Suthanthiran and colleagues
Abstract #1 ATC 2011
- Multicenter prospective serial urine collections on ~500 kidney transplant recipients
- qPCR for genes in urinary pellet RNA
  - Perforin, IP-10, CXCR3, granzyme B, others
- Preliminary analysis
  - Confirms diagnostic utility of gene expression profiling to diagnose AR
  - Suggests gene upregulation prior to clinical recognition of AR

Unbiased approaches to biomarker development
Genomics/Proteomics

Published studies of urine gene expression

<table>
<thead>
<tr>
<th>Genes (Ref)</th>
<th>Urinary Cytokine</th>
<th>Urinalysis *</th>
<th>Diag/Non</th>
<th>Odds ratio</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suthanthiran &amp; Col 2011</td>
<td>-</td>
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<tr>
<td>Brouard et al 2011</td>
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</tbody>
</table>

Unbiased approaches to biomarker development
Genomics/Proteomics

Gene profiling using arrays

Unbiased approaches to biomarker development
Genomics/Proteomics

Unbiased approaches to biomarker development
Genomics/Proteomics
3 mo protocol biopsy gene profiles predict ACR—GoCAR

- Normal bx and Prior ACR: 0
  - On biopsy: 3 borderline
  - Normal bx + Future ACR: 3
- Normal bx and Prior ACR: 1
  - On biopsy: 0 ACR
  - Normal bx + Future ACR: 0
- Normal bx and Prior ACR: 2
  - On biopsy: 0 ACR
  - Normal bx + Future ACR: 8
  \( p < 0.001 \)

B Murphy and colleagues

Discovery of a set of blood biomarkers for acute rejection in the blood?

- Sunil Kurian, Tony Mondala, Steve Horvath, Peter Langfelder, Traver Hart, Steve Head, Lana Schaffer, Katie Podshivalova, Eileen Wang, Lauren Aarreberg, and Terri Gelbart

Study Design (117 patients/5 Centers)

- 51 kidney transplant patients with biopsy-proven acute rejection (AR)
- 42 kidney transplant patients with acute dysfunction and no rejection (ADNR)
- 24 control kidney transplant patients with well-functioning kidneys biopsied by protocol at 1 year
- Samples randomized into Test, Qualifying and Validation Cohorts

AR-specific Predictions

Bruce McManus, Vancouver BC Canada

PROOF Centre of Excellence

- Not-for-Profit Society established in 2008 through competitive federal funding
- Anchored at St. Paul's Hospital and hosted by the University of British Columbia in Vancouver, BC
- An International Community of Partners

Acute Heart Rejection Diagnostic Panel

- Novel genomic approaches
  - Blood AUC = 0.60
  - Biopsy AUC = 0.85
  - Biopsy-guided blood AUC = 0.83

Peter S. Heeger, MD
www.a-s-t.org
Molecular profiles of biopsies for cause after year 1 correlate with future graft loss

Unbiased molecular approaches
- Blood, urine, graft transcript profiles are promising biomarkers
- Prospective validations are ongoing through multiple approaches (Proof center, CTOT, others)
  - Will need to test whether results can effectively guide treatment to improve outcomes
- Strategies for Standardization and commercialization are emerging (some companies already selling products)

Unbiased Proteomic approaches
- Urine
- Serum/plasma
- Graft

Detection of urine proteins with SELDI-TOF-MS
- 5 µL urine per spot
- Laser
- 8 cm

High throughput
Resolution <15kDa
Quantification based on peak intensity
Ion suppression
Resolution >15kDa
Lower sensitivity

Blood Cell Proteomics for Acute and Chronic Rejection: MudPIT, SRM and Triple Quad MS/MS
- Dan Salomon, Scripps
  - Alex Nakorchevsky, Steve Horvath
  - John Yates

Definition of the normal and rejection pattern
Proteolytic peptides of protein targets combined with the heavy labeled peptide analogues – SID-MS

Single reverse phase chromatography

Selected reaction ion monitoring (SRM) approach using a triple quadrupole mass spectrometer. SRM analysis with a triple quadrupole instrument has a dynamic range of $10^6$, meaning we can detect proteins ranging in abundance from $10^6$ to few copies per cell.

Acute Rejection Proteomics: Validation with Selected Reaction Monitoring (SRM) and Stable Isotope Dilution (SID)

ThermoScientific TSQ Vantage

Canonical Pathways
- Granzyme B Signaling
- Antigen Presentation Pathway
- Granzyme A Signaling
- DNA Methylation and Transcriptional Repression Signaling
- Systemic Lupus Erythematosus Signaling
- EIF2 Signaling
- IL-4 Signaling
- Allograft Rejection Signaling

Biomarkers in transplantation
- Define the endpoint
- Continue discovery
- Prospective validations of many markers are ongoing
- Standardization and commercialization are challenges that can be overcome

Towards Clinical Implementation

Biomarker discovery

- A panel of 24 genes discriminate acute rejection versus no rejection in kidney transplant patients
- A panel of 12 proteins discriminate acute rejection versus no rejection

Towards Clinical Implementation

Biomarker discovery

Bruce McManus, Vancouver BC Canada

Results of MudPIT Discovery: Proteins unique and upregulated in PBLs - AR

Paul Tanaka

Acute unique 705 proteins

Diff expressed 431 proteins

Healthy unique 761 proteins

Proposed monitoring strategy

Transplant Day 0

Pretransplant Risk assessment

HLA
Alloantibodies
T cell immunity (ELISPOT)
Genetic profiling
Implantation biopsy

Biopsy 6 mo

GFR 12 mo

Posttransplant monitoring

HLA
Alloantibodies
T cell immunity (ELISPOT)
Urine PCR
Urine Chemokines
Blood PCR

Individualized immunosuppression
Acknowledgements

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- Kanya Ostrów
- Paulina Trzcińska
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- Jennifer Smar
- Sam Gavzy
- Dean Firkus
- Maria Krasilinkova
- Will van der Torw
- Martin Lin, PhD
- Parth Lakhani
- Jordi Ochando, PhD

THANK YOU
Fellows Symposium on Transplantation Medicine

Sunday, September 25
9:05 am - 9:40 am

Tolerance Mechanisms in Human Trials

Kenneth A. Newell, MD, PhD

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org
Tolerance Mechanisms in Human Trials

AST Fellows Symposium 2011
Kenneth A. Newell
Emory University

Disclosures – K. A. Newell
- Biogen Idec - no impact on this presentation
- Grant support
- Intellectual property related to biomarkers of tolerance to be discussed

Outline
- The definition of tolerance
- Is tolerance important?
- Lesions from rodent and NHP studies of tolerance
  - Immune mechanisms producing tolerance
  - Barriers to tolerance
- Immune monitoring assays
- Summary of previous and ongoing studies of tolerance in humans

Definition of tolerance
- Experimental definitions of tolerance can not be applied to clinical transplantation
  - Long-term survival of a well-functioning allograft without immunosuppression in an immunocompetent recipient
  - No histologic evidence of rejection
  - In vitro and in vivo unreponsiveness to donor but not 3rd party organs/tissues/cells
- A working definition for functional or operational tolerance in humans would be continued stable allograft function in the absence of immunosuppression

The scope of transplantation 2010
- 20,000 organ transplants performed in 2009
- 85,000 individuals with end stage organ disease on the transplant waiting list
- 170,000 individuals living with a transplanted organ in the US

Virtually all take maintenance immunosuppression

Original clinical tolerance
  - 6 patients off IS
    - 4 maintained good function for at least 17-52 mos.
    - 2 with rejection; 1 graft lost
  - Survey of 40 transplant centers
    - 24 patients off IS
      - 22 patients had IS restarted; 2 remained off IS 9-36 mos
    - Recommended in absence of rejection not restarting immunosuppression
Same findings different conclusion
- Survey 165 US transplant programs
  - 48 off all IS
    - DDRT – 9 of 16 failed at a mean of 59 days
    - LDRT – 12 of 32 failed at a mean of 234 days
    - Of the 20 surviving 5 had function > 1yr and 6 > 3 years
- Emphasize that cessation of IS is not safe at any time after transplantation
- Those who have stopped should restart IS
  - Exception may be those doing well > 3 yrs off IS

Ongoing concerns vis-à-vis tolerance
Concerns raised 1. are tolerant patients immunocompetent
2. Lack of data that tolerance regimens improve long-term outcomes
3. Concerns about tacitly encouraging non-adherence

EMORY TRANSPLANT CENTER

Cadaveric Renal Allograft Survival
"Better living through pharmacology?"

EMORY TRANSPLANT CENTER

Outcomes of extra-renal transplants over time
Lodhi SA et al, AJT 2011;11:1226

EMORY TRANSPLANT CENTER

Stagnation of long-term outcomes in renal transplantation

EMORY TRANSPLANT CENTER

Chronic Renal Failure after Transplantation of a Nonsrenal Organ
Lodhi SA et al, AJT 2011;11:1226
Long-term benefits of withdrawing immunosuppression

- Tryphonopoulos and Tzakis et al. (ATC 2008 Poster 1367 P211-III)
- 104 liver transplant recipients underwent planned attempts at weaning immunosuppression
  - No autoimmune disease
  - > 3 yrs post-transplant
  - Stable function for > 1yr
- 20 successfully weaned compared to 73 on immunosuppression

Benefits of IS withdrawal

- No difference in patient survival or rejection
- Decreased risk of CA and infections
  - 1/20 (5%) off IS
  - 10/73 (13.7%) on IS

Benefits of tolerance (2)

- Study of drug withdrawal vs. maintenance IS in liver transplantation for HCV
  - Similar 10 yr survival and histologic injury on protocol biopsy
  - Less NODAT, cardiovascular disease, and fewer infections in tolerant group
  - Lower HCV viremia in tolerant group

Limitations of current regimens

- Promote non-compliance
- Increase risks of cardiovascular disease
  - Hypertension
  - Post-transplant diabetes mellitus
  - Cholesterol/tipid abnormalities
- Nephrotoxicity/fixd allograft survival
  - Increased demands on the waiting list
  - Economic costs of dialysis/re-transplantation
- Over-immunosuppression
  - Infections
  - Malignancies

Does tolerance exist?

- TM – LRRT from haploidentical donor 1975
- Prednisone and Imuran
- Discontinued in 1981 due to side-effects
- Cr 3/21/05 0.9 mg/dL without proteinuria
Is tolerance permanent?

- AB – glomerulonephritis at age 14
- First transplant DDRT 1973
- Lost due to vascular problems
- Second transplant LRRT from brother 1983
- Prednisone (weaned 2000) and Imuran (weaned 2003) by nephrologist
- Cr 1.0 – 1.3 mg/dL in 2004, 1.9 mg/dL at Emory 8/25/05
- Currently taking Rapamune 2 mg/day with Cr of 1.4 mg/dL

Tolerance is metastable

My conclusions

- Tolerance remains an important goal to:
  - Avoid the toxicities of chronic immunosuppression
  - Tolerance/tolerant patients do exist and are not all immunoincompetent
  - Tolerance is not necessarily permanent or stable
  - Despite the "self-evident" benefits associated with tolerance, trials comparing tolerance to best current immunosuppressive regimens will be necessary

The legacy of Sir Peter Medawar

- Experimental design
  - Crude cell/tissue mixture from an allogeneic adult mouse injected into 6 fetuses of a CBA female produced 5 pups
  - Pups skin-grafted at 8 weeks
  - Experiments repeated in neonatal mice
- Results
  - Of 5 skin grafts
    - 2 promptly destroy (likely acute rejection)
    - 1 with prolonged involution (likely chronic rejection)
    - 2 accepted at 77 and 101 days
  - Implantation of LN fragments from donor antigen-immunized mice lead to prompt rejection of these two surviving grafts
  - Inoculation of neonatal mice with various tissues of different strains was largely unsuccessful

Sir Peter Medawar - conclusions

- Medawar and colleagues
  - Effect of treatment was a continuum ranging from no effect on survival to indefinite survival
  - Regimen ineffective in neonatal (older) mice
  - Therapy consistently overcome by memory
- Our misconceptions
  - A brief, limited intervention in most individuals can consistently produce tolerance to alloantigens

Early demonstration of suppression

Blockade of CD154 and CD28 prevents rejection

Larsen et al. Nature 381:434, 1996

Tolerance mechanisms
- Ignorance
  - Absence of secondary lymphoid organs
  - Immune privileged sites
- Clonal exhaustion
- Anergy
- Regulation
- Deletion
  - Specific deletion of donor-reactive cells
  - Non-specific depletion of donor-reactive cells
- Note: mechanisms may change over time or vary by organ

Barriers to tolerance
- Size of the alloreactive repertoire
- Frequency of TCR seeing nominal antigen is 1 in $10^5$ – $10^6$
- Frequency of TCR seeing alloantigens is 1 in 10 to $10^3$
- Memory
  - Heterologous immunity
    - Adams et al J Clin Invest 2003; 111:1887
  - Homeostatic proliferation
    - Wu et al. Nat Med 2004; 10:87

Characteristics of the Adult Naïve T cell repertoire

<table>
<thead>
<tr>
<th></th>
<th>T cell #</th>
<th># unique TcR(clones)</th>
<th>Clone size</th>
<th># alloreactive T cells</th>
<th># Alloreactive TcR(clones)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^9</td>
<td>2 x 10^5</td>
<td>1-4 x 10^5</td>
<td>100-200</td>
<td>1-2 x 10^9</td>
</tr>
<tr>
<td></td>
<td>2 x 10^8</td>
<td>2 x 10^6</td>
<td>2 x 10^6</td>
<td>1-2 x 10^6</td>
<td>1-2 x 10^9</td>
</tr>
</tbody>
</table>

Targeting memory T cells
- CD4 and CD8 memory T cells express CD2
- Alefacept (LFA3-Ig) binds CD2 and depleted memory T cells
- LFA3-Ig, CTLA4-Ig, sirolimus, and DST promotes long-term graft survival

Weaver Nat Med 2009;15:746

T cell tolerance & B cell tolerance
- 5 patients with bone marrow and kidney from a haploidentical living donor
- Conditioning with cyclophosphamide, ATG, thymic irradiation and CSA weaned to off
- 1/5 irreversible humoral rejection
- 4 weaned from IS at 9 – 14 mos
- Display tolerance in the T cell compartment
  - T cell hyporesponsiveness to donor in vitro
  - Evidence of B cell immunity despite T cell tolerance
    - 2 of 4 with de novo donor-specific anti-HLA antibodies
    - Associated with increased numbers of transitional B cells
    - Preceded by elevated serum BLyS levels

Kenneth A. Newell, MD, PhD
www.a-s-t.org
**PRA ≠ panel reactive T cells**

- Study of 41 hemodialysis patients
  - Positive result > 25 spots/300,000 PBL
  - +PRT defined as positive result with 40% or 75% of all stimulators
- Results
  - 17% PRT 75+
  - 32% PRA+
  - 12% PRT-75+/PRA-
  - 5% PRA-75+/PRA+
- Conclusion: T cell and B cell sensitization do not correlate directly

By Poggio et al., JASN 2005

**Hierarchy of Immunogenicity**

- Liver (highest) → Kidney → Heart → Islets → Pancreas → Intestine → Skin (lowest)

By Zhang et al., Transplantation 1996;62:1267

**Assays associated with tolerance**

<table>
<thead>
<tr>
<th>Tissue Assayed</th>
<th>Result in Tolerance</th>
</tr>
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<tbody>
<tr>
<td>Tissue</td>
<td>Assay Type</td>
</tr>
<tr>
<td>Kidney</td>
<td>Flow phenotyping of B cells</td>
</tr>
<tr>
<td>Blood</td>
<td>Flow phenotyping of NK cells</td>
</tr>
<tr>
<td>Liver</td>
<td>Flow phenotyping of γδ cells</td>
</tr>
<tr>
<td>Blood</td>
<td>Flow phenotyping of γδ cells</td>
</tr>
<tr>
<td>Liver</td>
<td>Flow phenotyping of Plasmacytoid DC</td>
</tr>
<tr>
<td>Blood</td>
<td>Flow phenotyping of Plasmacytoid DC</td>
</tr>
<tr>
<td>Kidney</td>
<td>Flow phenotyping of CD8+CD28- T cells</td>
</tr>
<tr>
<td>Blood</td>
<td>Flow phenotyping of CD4+CD25hi T cells</td>
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<tr>
<td>Kidney &amp; Liver</td>
<td>Gene expression of B cell related genes</td>
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<tr>
<td>Blood</td>
<td>Gene expression of B cell related genes</td>
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<tr>
<td>Liver &amp; Kidney</td>
<td>Gene expression of NK cell related genes</td>
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<tr>
<td>Blood</td>
<td>Gene expression of NK cell related genes</td>
</tr>
<tr>
<td>Liver</td>
<td>Gene expression of γδ cell related genes</td>
</tr>
<tr>
<td>Blood</td>
<td>Gene expression of γδ cell related genes</td>
</tr>
<tr>
<td>Liver &amp; Kidney</td>
<td>Gene expression of FoxP3</td>
</tr>
<tr>
<td>Blood &amp; graft</td>
<td>Gene expression of FoxP3</td>
</tr>
<tr>
<td>Kidney</td>
<td>Cytokine production</td>
</tr>
<tr>
<td>Blood</td>
<td>Cytokine production</td>
</tr>
<tr>
<td>Kidney</td>
<td>Trans-vivo DTH</td>
</tr>
<tr>
<td>Blood</td>
<td>Trans-vivo DTH</td>
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</tbody>
</table>

**Studies in liver transplantation**

- Incidence of tolerance in selected groups approaches 20%*
  - Time since transplant; monotherapy; non-autoimmune, non-HCV etiology of ESLD
  - Study design largely planned withdrawal of IS in patients doing well on treatment with conventional IS agents

*By Lerut Am J Transplant 2006;6:1774

**Kyoto experience**

- 15% (87 of 581) pediatric recipients of living donor transplants (54 protocol and 33 EBV related)
  - Increased CD4+CD25+ and V61 IL-10 producing cells in blood and FoxP3+ cells in the graft
  - Suggests a mechanistic role for regulation
  - Late biopsies show increased fibrosis and decreased bile duct size

By Koshiba Transpl Int 2007;17:94 & Yoshitomi Transplantation 2009;84:606
Functional T cell changes identify tolerance

- Compared 13 liver transplant recipients weaned from IS to 11 developing rejection and 80 HV
- PBL stimulated with mitogens in vitro
- Cohort with rejection
  - Increased IFNγ and decreased TGFβ
  - Increased CD4+IFNγ and CD8+IL-2/IFNγ

Millan Clin Immunol 2010 [Epub ahead of print]

Results of ITN029ST

- 12 of 20 patients weaned over 8 – 12 mos
  - Off immunosuppression for 3 – 20 mos without evidence of rejection
- 4 failed weaning
  - 1 rejection treated with steroids
  - 1 inclusion criteria violation
  - 2 abnormal LFTs with non-diagnostic biopsy
- Allo and autoantibodies remain negative

Tolerance following pediatric liver transplantation

- ITN029ST (P.I. S. Feng; Clinicaltrials.gov identifier NCT00320606)
  - Centers – UCSF, Children’s Memorial Hospital (Chicago), and Children’s of New York-Presbyterian
  - 20 children enrolled between 5/06 and 7/08
  - Recipient of a living donor graft from parent > 4 yrs prior to enrollment
  - Stable function on CNI monotherapy
  - Screening biopsy – no rejection and minimal fibrosis
  - IS weaned over 8 months
  - Rejection or failure to wean for 4 weeks prompted termination

Tolerance in kidney transplantation

- Study of spontaneously tolerant recipients (rare)
- Strategies to promote tolerance
  - T cell depeletion – alemtuzumab
  - mTOR inhibition
  - Costimulation blockade
  - Hematopoietic cell infusion/transplantation
    - Without conditioning as a tool to induce anergy or clonal exhaustion
    - With conditioning with the aim of inducing mixed chimerism
Molecular differences define tolerant kidney transplant recipients

- 17 tolerant renal txp recipients
  - 49 genes distinguish tolerant pts from other groups
  - 33 highly predictive of tolerance
  - Decreased FoxP3 in CR vs. Tol and HC
  - TGFβ not different between groups but 27% of "tolerance genes" regulated by TGFβ
  - Decreased costimulatory molecules, activation/effectors molecules, cytolytic proteins and proinflammatory cytokines
  - Moderate increase in memory T cells


Combined bone marrow and solid organ transplantation

- 6 patients with multiple myeloma
  - Received BM and kidney from HLA identical donor
  - Conditioning with cyclophosphamide, ATG, thymic irradiation and CSA weaned to off
  - All with long-term functioning kidney
    - 3/6 transient chimerism; off IS
    - 1/6 with rejection; IS re-introduced temporarily

Kawai et al. NEJM 358:353; 2008

Sirolimus Monotherapy in Kidney Transplantation: Results of an ITN-Sponsored Trial (S. Knechtle, Univ. of Wisconsin)

- Well matched: at least 3/6 antigen match; no HLA identical
- 10 patients: 9 LRD, 1 DD kidney transplant

Am J Transplant 2009; 9:1087-1098

Treatment Protocol

Day of transplant
Day 0 1        2 60 365
Campath-1H 30 mg i.v.
Sirolimus 2 mg/d Tacrolimus 2 mg BID Tacrolimus discontinued
Biopsy, Assays; Possible Sirolimus withdrawal

Weaning criteria at 12 months
- No clinical or biopsy evidence of rejection
- GFR>50 ml/min
- Consent to withdrawal

Sirolimus Monotherapy

- Monotherapy
  - in Kidney Transplantation: Results of an ITN-Sponsored Trial
  - (S. Knechtle, Univ. of Wisconsin)
  - Well matched: at least 3/6 antigen match; no HLA identical
  - 10 patients: 9 LRD, 1 DD kidney transplant

Am J Transplant 2009; 9:1087-1098

12 Month DSA, CKT, TV-DTH

<table>
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<tr>
<th>Subject</th>
<th>DSA</th>
<th>CKT</th>
<th>TV-DTH</th>
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<td>regulator</td>
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<td>2</td>
<td>anti-B52, DR13</td>
<td>hyper</td>
<td>no reg</td>
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<tr>
<td>3</td>
<td>neg</td>
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<td>regulator</td>
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<td>hypo</td>
<td>regulator</td>
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<tr>
<td>5</td>
<td>anti-B37</td>
<td>hypo</td>
<td>no reg</td>
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<td>6</td>
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<td>7</td>
<td>weak anti-A3</td>
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<tr>
<td>10</td>
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<td>no reg</td>
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</tbody>
</table>

No rejections during weaning

Kenneth A. Newell, MD, PhD
www.a-s-t.org
American Society of Transplantation
 Fellows Symposium

September 23-25, 2011
Grapevine, TX

ITN study of tolerant kidney transplant recipients

<table>
<thead>
<tr>
<th>Immune Tolerance Network</th>
<th>University of Wisconsin</th>
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<tbody>
<tr>
<td>Adam Azari</td>
<td>Lynn Haynes</td>
</tr>
<tr>
<td>Richard Wang</td>
<td>Eva Gan</td>
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<tr>
<td>Zhong Gao</td>
<td>Bill Buringham</td>
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<tr>
<td>Vicki Seyfert</td>
<td>Swedish Medical Center</td>
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<tr>
<td>Karla Bourdelier</td>
<td>Josh Ulmeron</td>
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<td>Trang Giller</td>
<td>Bill Marks</td>
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<td>University of Rochester</td>
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<td>Amy Lewis</td>
<td>Ignacio Sainz</td>
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<tr>
<td>Elizabeth Ford</td>
<td>Beth Israel Deaconess</td>
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<tr>
<td>Allan Kirk</td>
<td>Lawrence Turks</td>
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<td>Roz Mannen</td>
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</tbody>
</table>

King’s College, London UK
Maria Hernandez-Fuentes and Robert I. Lecher

Potential Tolerance Footprints by Array

30 transcripts differentially expressed between TOL and SI at >2 FC, p <0.05.
22/30 are B cell specific

Primarily up-regulated in TOL group compared to SI

Array: HG-U133 Plus 2.0 Affymetrix Mississauga – 54K transcripts

B cell transcripts and tolerance

Tolerance was associated with cell surface staining for markers of naïve B cells, NK cells and HLA-DR+ CD4 T cells

Quantitative Gene Expression by PCR

30 transcripts differentially expressed between TOL and SI; p <0.05,
17/30 also found in microarray
26/30 are B cell specific

Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Tol – training</th>
<th>Tol – test</th>
<th>Stable – IT</th>
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<td>(n=33)</td>
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<tr>
<td>Donor type</td>
<td>4 cadaveric, 14 live, 1 unknown</td>
<td>1 cadaveric, 4 live, 1 unknown</td>
<td>5 cadaveric, 23 live, 2 unknown</td>
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<tr>
<td>Race</td>
<td>18 white, 1 Asian</td>
<td>6 white</td>
<td>28 white, 4 AA, 1 Asian</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1 (0.6 – 1.8)</td>
<td>0.95 (0.7 – 1.5)</td>
<td>1.4 (0.7 – 2.8)</td>
</tr>
<tr>
<td>Years post-Tx</td>
<td>20 (7 – 40)</td>
<td>12 (5-20)</td>
<td>5 (1-40)</td>
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<tr>
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<td>5.5</td>
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Patient demographics

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Urine PCR distinguishes TOL from SI and HC

Transitional B cells identify tolerance

CD19+CD24+CD28+CD122+IgD-

IL-10 and TGFβ production by transitional B cells

Immune Tolerance Fingerprint in Renal Transplants

Project Co-ordinator: Dr. Maria Hernandez-Fuentes

CoPIs: Dr Anthony Warrens, Dr Uwe Janssen, Prof. Michel Goldman, Prof. Kathryn Wood, Prof. Hans-Dieter Volk, Prof. Jean-Paul Soulillou

Principal Investigator: Prof. Robert Lechler

Patient Recruitment

<table>
<thead>
<tr>
<th>HC</th>
<th>Tol-DF</th>
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<th>s-nCNI</th>
<th>S-CNI</th>
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<tr>
<td>Age</td>
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<td>54</td>
<td>49</td>
<td>50</td>
<td>42</td>
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<tr>
<td>% Female</td>
<td>47</td>
<td>18 *</td>
<td>33</td>
<td>40</td>
<td>48</td>
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<tr>
<td>Ys Post-Tx</td>
<td>12</td>
<td>14</td>
<td>25 *</td>
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*P < 0.05 Mann-Whitney vs All patients
Diagnostic capabilities of combined biomarkers

- 9 most significant genes
- Ratio of B/T subsets
- Percentage CD25+CD4+ (Ratio of anti-donor to anti-3rd party ELISPOT)

Specificity = 0.964
Sensitivity = 0.933
PPV = 82.4%
NPV = 98.7%

All calculated within sample classification

Overlap in gene signatures in 3 studies - B cell-related genes*

Stability of the B cell signature over time

Lack of overlap in kidney and liver tolerance signatures

Tolerance in kidney ≠ liver
**B cell phenotype & tolerance**

Liu Nat Med 2007;13:1295

- Following depletion of T and B cells a subset of islet allografts in NHPs survive long-term
- Long-term survival is associated with recovery of transitional rather than memory B cells

**B cell repertoire remodeling**

- Noorhashm, Parsons and Naji have proposed that long-term allograft acceptance will require remodeling of the B cell repertoire
- B cell depletion at the time of transplant
- Presence of donor antigen as B cells re-emerge (preferably as hematopoietic cells)
- Auto-deletion of graft-reactive B cells as occurs for self antigens in normal B cell repertoire development


**Tolerance to renal allografts - A rational approach**

- PT Allan Kirk (clinicaltrials.gov identifier NCT00565773, ATC 2011 Abstract 56)
- Targeted enrollment 20 patients
  - T cell depletion – alemtuzumab
  - Costimulation blockade – belatacept
  - Transient mTOR inhibition – sirolimus
  - 10 patients receive donor BM infusion
  - Spaced weaning of sirolimus at 1 yr and belatacept at 2 yrs

**Results – ATC 2011 Abstract 56**

Sunday May 1 Room 204C 3:03 PM

- Rejection*: 1 of 19 with early rejection day 10 (responded to 3 day pulse of CS)
- 11 protocol biopsies at 1 yr: 2 Banff grade 1 subclinical rejections
- No alloantibodies detected*
- BK (n=7) & EBV (n=4) viremia (↓ IS)
- Repertoire repopulation: increased FoxP3+CD4+CD25+ & transitional type B cells

**B cells promote tolerance**

Treatment with DSG analogue induces tolerance in rat heart txpl model

1. Grafts infiltrated by B cells
2. B cells blocked at IgM to IgG switch
3. Increased expression of BANK-1 and inhibitory receptor FcγR2b
4. Tolerance transferred with B cells from tolerant recipients


**B cells & tolerance at ATC 2011**

- Abstract #204 (Cherukuri et al): alemtuzumab induces increased Breg relative to basiliximab in humans
- Abstract #245 (Lai et al): depletion of B (reg) cells induces IL-6 dependent CCR6+ Th17 mediated rejection
- Abstract #246 (Ding et al): TIM-1/TIM-4 ligation → IL-10 enriched TIM-1+B reg and↑ graft survival
- Abstract #1154 (Kim et al): ↑ survival induced by anti-CD45 mAb is dependent upon Breg (adoptive transfer)
- Abstract #1667 (Chen et al): IL-10 producing B cells are necessary to anti-CD40 + DST induced tolerance
Conclusions

- Tolerant patients do exist
- Carefully supervised weaning of IS can be safely performed – particularly following liver transplantation
- Weaning of IS may result in decreased morbidity and excellent long-term outcomes
- Several studies have identified promising candidate markers, including NK cells (liver) and B cells (kidney), that may identify or predict the tolerant state
Fellows Symposium on Transplantation Medicine

Sunday, September 25
10:00 am - 11:00 am

Literature in Transplantation: Key Papers of 2010 - 2011
Combined Session

There are no advance slides for this session.

September 23-25, 2011
Hilton DFW Lakes Executive Conference Center
Grapevine, Texas
www.a-s-t.org
Fellows Symposium on Transplantation Medicine

Sunday, September 25
11:00 am – 12:15 pm

Career Choices in Transplantation

**Clinical Research Track:** John S. Gill, MD, Basic Science and Translational Track: Peter S. Heeger, MD and Scott M. Palmer, MD, MHS, **Careers in Liver and Thoracic Transplantation:** Mark L. Barr, MD, Kimberly A. Brown, MD, Maryl R. Johnson, MD, and Shawn J. Pelletier, MD, **Clinical Transplantation:** Michelle A. Josephson, MD and Milagros D. Samaniego, MD

There are no advance slides for this session.

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