The transcriptome of the renal transplant biopsy: the lessons

Philip F Halloran

Alberta Transplant Applied Genomics Centre
April 27th 2009

Congratulations on your 50th anniversary
Learning objectives: mechanisms and applications

• Rethinking how diagnoses are made
• What is T-cell mediated rejection (TCMR)?
• What is antibody mediated rejection (ABMR)?
• What causes progression to organ failure?
  — a lesson for primary kidney disease
Key points about studying biopsies

• Diagnostic labels are based on non specific features plus context
• Existing diagnostic labels are arbitrary and inaccurate
  — record features e.g. lesions
• Molecules are non specific features, to be understood in relationship to other features and context
• All features (molecules, lesions, clinical) reflect the same underlying biological processes
• Leave theories behind: let the data speak
Acknowledgements

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Canada Research Chair in Life Sciences

Special thanks to our clinical collaborators

Special thanks to our patients
Contents

• The transcriptome project
• Diagnoses are based on non specific features
• Changes in diseased tissues are stereotyped
• Reassessing T cell mediated rejection
• Reassessing antibody mediated rejection
• Determinants of progression to failure
• Summary
The transcriptome project

Diagnoses are based on non-specific features

Changes in diseased tissues are stereotyped

Reassessing T cell mediated rejection

Reassessing antibody mediated rejection

Determinants of progression to failure

Summary
The Edmonton Genome Canada Project

The transcriptome: our new microscope
The Edmonton Genome Canada Project

Three goals:
1. Understand mechanisms
2. Use molecular findings to improve histopathology
3. Diagnostic molecular tests

The transcriptome: our new microscope
This strategy generates a feature matrix

<table>
<thead>
<tr>
<th>Features:</th>
<th>Molecules</th>
<th>Molecule herds</th>
<th>Pathology lesions</th>
<th>Pathology Diagnoses (often wrong)</th>
<th>ID data e.g. Virus load</th>
<th>PRA DSA</th>
<th>Clinical phenotype</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecules</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Molecule herds</td>
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<td>Pathology lesions</td>
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<td>Pathology diagnoses</td>
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<td>PRA DSA</td>
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<tr>
<td>Clinical phenotype</td>
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<td>Outcomes</td>
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<td>X</td>
</tr>
</tbody>
</table>
Maximizing the granularity of conventional data: kidney transplant biopsy lesions

- C4d 0, focal vs diffuse
- ptc 0 1 2 3
- g 0 1 2 3
- cg 0 1 2 3
- mm 0 1 2 3
- ptcbmmml 0 1 2 3
- t 0 1 2 3
- i 0 1 2 3
- v 0 1 2 3
- ct 0 1 2 3
- ci 0 1 2 3
- cv 0 1 2 3
- ah 0 1 2 3
- PRA DSA any class I, class II; quantify
Key features of our study

- Prospective, consent-based
- Inclusive, unselective
- Complete: includes PRA/DSA, electron microscopy
- Based on clinical phenotypes and outcome events: not protocol biopsies
- Maximizes granularity of lesion data
The biopsy for cause population

Different from other populations
Includes all late failures
Indications: dysfunction, proteinuria
## Clinical status at time of biopsy (N=234)

<table>
<thead>
<tr>
<th></th>
<th>All biopsies</th>
<th>Early (1y)</th>
<th>Late (&gt;1y)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time post transplant</strong> (months)</td>
<td>43 ± 60</td>
<td>3.8 ± 3.4</td>
<td>75 ± 65</td>
</tr>
<tr>
<td><strong>Patient age</strong> (years)</td>
<td>49 ± 15</td>
<td>51 ± 16</td>
<td>47 ± 14</td>
</tr>
<tr>
<td><strong>Renal function</strong> (eGFR ml/min)</td>
<td>44 ± 19</td>
<td>42 ± 16</td>
<td>47 ± 18</td>
</tr>
<tr>
<td><strong>Proteinuria</strong> (% present) [n = 226]</td>
<td>115 (51%)</td>
<td>48 (47%)</td>
<td>67 (54%)</td>
</tr>
<tr>
<td><strong>Antibody status</strong>[n = 206]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA class I positive</td>
<td>33 (16%)</td>
<td>14 (15%)</td>
<td>18 (16%)</td>
</tr>
<tr>
<td>PRA class I+II positive</td>
<td>39 (19%)</td>
<td>4 (4%)</td>
<td>35 (31%)</td>
</tr>
<tr>
<td>PRA class II positive</td>
<td>34 (17%)</td>
<td>6 (6%)</td>
<td>28 (25%)</td>
</tr>
<tr>
<td>PRA negative</td>
<td>100 (49%)</td>
<td>70 (74%)</td>
<td>31 (28%)</td>
</tr>
</tbody>
</table>
Contents

• The transcriptome project
• *Diagnoses are based on non specific features*
• Changes in diseased tissues are stereotyped
• Reassessing T cell mediated rejection
• Reassessing antibody mediated rejection
• Determinants of progression to failure
• Summary
Diagnostic labels are unstable: they are only estimates of the probability of a disease.

*The Truth*
true disease process
(Mechanisms of injury
response to injury
consequences)

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Function</th>
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<tbody>
<tr>
<td>Signs</td>
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<tr>
<td>Laboratory tests</td>
<td></td>
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<tr>
<td>Imaging e.g. MRI</td>
<td></td>
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</tbody>
</table>

| Outcomes    |          |

Function
Diagnostic labels are unstable:
they are only estimates of the probability of a disease

Tissue biopsy read by histopathology

The Truth
true disease process
(Mechanisms of injury
response to injury
consequences)

Symptoms
Signs
Laboratory tests
Imaging e.g. MRI

Function
Outcomes
Diagnostic labels are unstable: they are only estimates of the probability of a disease.

- **The Truth**: true disease process (Mechanisms of injury, response to injury, consequences)
- **Symptoms**
- **Signs**
- **Laboratory tests**
- **Imaging e.g. MRI**
- **Function**
- **Outcomes**

**Tissue biopsy** read by histopathology

- Assess features (“lesions”) (arbitrary rules)
  - Inflammation, etc

- Assign “diagnosis” (label) (arbitrary rules based on context)
  - Define probability of a disease
Diagnostic labels are unstable: they are only estimates of the probability of a disease.

- **Tissue biopsy** read by histopathology
- **Assess features ("lesions")** (arbitrary rules)
  - Inflammation, etc
- **Assign "diagnosis" (label)**
  - Arbitrary rules based on context
  - Define probability of a disease

---

**50-70% Accuracy?**

- **The Truth**
  - True disease process
  - (Mechanisms of injury response to injury consequences)

---

- **Symptoms**
- **Signs**
- **Laboratory tests**
- **Imaging e.g. MRI**

- **Function**

- **Outcomes**
Inflammation, Tubulitis
Glomerulonephritis in a native kidney showing tubulitis and interstitial inflammation

Moderate to marked tubulitis and interstitial inflammation in glomerulonephritis
Lesions are objective but not specific

- Tubulitis, interstitial infiltrate occur in primary renal diseases: e.g. ATN, GN
- Tubulitis occurs in ABMR (t1,2)
- Glomerulitis (g) and endothelialitis in small arteries (v) occur in ABMR and TCMR
The three main types of change in a biopsy for cause

- Sick versus well (“disturbance in the force”)
- Specific disease states
  - Rejection vs non rejection
  - TCMR vs ABMR
  - Specific disease definitions
- Is this organ at risk for failure?
The three main types of change in a biopsy for cause

• Sick versus well ("disturbance in the force")
• Specific disease states
  – Rejection vs non rejection
  – TCMR vs ABMR
  – Specific disease definitions
• Is this organ at risk for failure?

Molecules detect the same types of change as lesions
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• Molecular changes in diseased tissues are stereotyped
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Molecules move in herds
pathogenesis-based transcript sets (PBTs)

No molecule changes alone in a biopsy
The concept of pathogenesis based transcript sets (PBTs)

• Concept: altered expression of any gene in a disease state is part of a **PBT** reflecting a biological event

• PBT is not just a collection of genes but the fingerprint of a biological event
  
  – e.g. a cytotoxic T lymphocyte has GzmB mRNA but MUST have mRNA for granulysin, CD3, etc
Comparing Microarray Versus RT-PCR Assessment of Renal Allograft Biopsies: Similar Performance Despite Different Dynamic Ranges

Kara Allanagh1, Michael Mustepe2, Danielle Bowers-Bendix2, Brian Meades1, G. Hide Thompson2, Michael Morrissey1, and Philip J. Halloran2

1Department of Medicine, Division of Nephrology, University of Alberta, Edmonton, Canada
2Department of Pathology, University of Alberta, Edmonton, Canada

Corresponding author: Philip J. Halloran, phil.halloran@ualberta.ca

Introduction

The objective of this study was to compare the performance of microarray versus real-time PCR (RT-PCR) techniques for detecting and quantifying gene expression in renal allograft biopsies.

Expression of B Cell and Immunoglobulin Transcripts Is a Feature of Inflammation in Late Allograft Rejection

Josephine Jackson, Scott M. Wong, Steven A. Frager, and Mark J. D'Angio

Introduction

Allograft rejection is an immune response that generates effector T lymphocytes (CD4+ and/or CD8+), as well as B lymphocytes and plasma cells that secrete immunoglobulins (Igs). The expression of B cell and immunoglobulin transcripts has been associated with allograft rejection, but the role of these transcripts in the pathogenesis of chronic allograft rejection is not well understood.

The Transcriptional Profile of Human Kidney Cytotoxic T Cells: Similarities and Disparities Among Allogeneic CD4+ CTL, CD8+ CTL, and NK Cells

L. G. Hildago1, C. Eischeid2, K. Allanagh2, and Philip J. Halloran2

Introduction

The transcriptional profile of human kidney cytotoxic T cells (CTLs) is thought to be important in the rejection of renal allografts. However, the transcriptional profile of these cells is not well characterized.

The Transcriptome of CD103+ Perforin1+ T Cells in the Kidney: Expression of Novel Transcripts During Allograft Rejection

G. Eiteke1, T. Fialkhead1, L. G. Hidalgo1, B. Sies1, F. Turner1, L.-F. Zhu2, R. C. Bleackley1, G. A. Haslett1, K. S. Famulski3, and P. F. Halloran1

1Department of Medicine, Division of Nephrology and Transplantation Immunology, University of British Columbia, Vancouver, Canada
2Department of Pathology, University of British Columbia, Vancouver, Canada
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Introduction

The objective of this study was to identify novel transcripts expressed in CD103+ Perforin1+ T cells during allograft rejection.

Microarray Analysis of Rejection in Human Kidney Transplant Using Pathogenesis-Based Transcript Sets

T. J. Mustepe1, G. Eischeid2, B. Sies1, F. Turner1, L.-F. Zhu2, R. C. Bleackley1, G. A. Haslett1, K. S. Famulski3, and P. F. Halloran2

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The objective of this study was to identify novel transcripts expressed in CD103+ Perforin1+ T cells during allograft rejection.

Conclusion

The use of microarray and RT-PCR techniques in renal allograft biopsies is a powerful tool for detecting and quantifying gene expression. The results of this study suggest that the two techniques have similar performance in detecting rejection-related gene expression.

The transcriptional profile of human kidney cytotoxic T cells is complex and involves both CD4+ and CD8+ T cells, as well as NK cells. The expression of genes involved in the immune response is upregulated in these cells, indicating their involvement in the rejection process.

The identification of novel transcripts expressed in CD103+ Perforin1+ T cells during allograft rejection can lead to a better understanding of the pathogenesis of chronic allograft rejection.

Expression of B Cell and Immunoglobulin Transcripts is a Feature of Inflammation in Late Allograft Rejection

Allograft rejection is an immune response that generates effector T lymphocytes (CD4+ and/or CD8+), as well as B lymphocytes and plasma cells that secrete immunoglobulins (Igs). The expression of B cell and immunoglobulin transcripts has been associated with allograft rejection, but the role of these transcripts in the pathogenesis of chronic allograft rejection is not well understood.

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The identification of novel transcripts expressed in CD103+ Perforin1+ T cells during allograft rejection can lead to a better understanding of the pathogenesis of chronic allograft rejection.
Translated mouse pathogenesis based transcript sets (PBTs) to corresponding human transcripts (orthologs)
PBT approach has two advantages

- Estimating biologic processes operating
- Annotating molecules defined by biostatistics
Pathogenesis-based transcript sets (PBTs)

<table>
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<tr>
<td>Cytotoxic T cells (CATs)</td>
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<td>IFN-g effect (GRITs)</td>
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<tr>
<td>Unstimulated macrophages</td>
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<tr>
<td>Classical macrophage activation</td>
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<tr>
<td>Alternative macrophage activation (AMA)</td>
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<tr>
<td>Renal transcripts induced in injury</td>
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<tr>
<td>• intermediate peak</td>
</tr>
<tr>
<td>• Late peak</td>
</tr>
<tr>
<td>Kidney transcript loss in injury (KTs)</td>
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<td>Endothelial injury, remodelling (ENDATs)</td>
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Probe sets for each transcript sets are available at http://transplants.med.ualberta.ca/.
## Pathogenesis-based transcript sets (PBTs)

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Biopsies for cause ordered by CTL associated transcripts

T Mueller G Einecke et al. AJT 7:2712, 2007

CATs, GRITs, KTs in human kidney biopsies for cause

Biopsies for cause ordered by CTL associated transcripts
At the transcript level ABMR resembles TCMR e.g. GZMB, IFNG effects

T Mueller G Einecke et al. AJT 7:2712, 2007
Most molecular changes and histologic features are probably non-specific consequences triggered by the causative mechanisms.

Other stimuli: e.g. ischemia, viruses, primary diseases

Cognate antigen-specific immune recognition
- CTL
- Alloantibody

Parenchymal change and final common pathway
- Parenchymal dedifferentiation: “Functio Laesa”
- Matrix remodelling, TGFB, endothelin effects
- Re-emergence of embryonic pathways

Stereotyped injury/inflammation compartment
- Recruit and activate macrophages/dendritic cells
- Recruit CTL and Effector-Memory T cells (not NKs)
- Late: B cells, plasma cells, Treg
• The transcriptome project
• Diagnoses are based on non specific features
• Changes in diseased tissues are stereotyped
• *Reassessing T cell mediated rejection*
• Reassessing antibody mediated rejection
• Determinants of progression to failure
• Summary
The rejection process

T cell mediated rejection
The rejection process

T cell mediated rejection

Host and donor antigen presenting cells move to lymphoid organs
The rejection process

- T cell mediated rejection
- Host and donor antigen presenting cells move to lymphoid organs
- Antigen presenting cells meet T cells in lymphoid organs
- T cell activation
The rejection process

T cell mediated rejection

Host and donor antigen presenting cells move to lymphoid organs

Antigen presenting cells meet T cells in lymphoid organs

T cell activation

Effector T cells home to graft
The rejection process

T cell mediated rejection

Host and donor antigen presenting cells move to lymphoid organs

Antigen presenting cells meet T cells in lymphoid organs

T cell activation

Effect T cells home to graft
Defining canonical TCMR (kidney)

- Transcripts: T cell burden, IFNG effects, alternative macrophage activation
- Highest t, i scores
  - (v can be either TCMR or ABMR)
- Functional disturbance
- Responds well to therapy, graft loss rare
- *Canonical TCMR is rare in protocol biopsies*: t, i in protocol biopsies are usually non-specific and do not indicate true TCMR
• The transcriptome project
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• **Reassessing antibody mediated rejection**
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Antibody mediated rejection features:
Microcirculation changes, C4d and donor specific antibody

Host and donor antigen presenting cells move to lymphoid organs

Antigen presenting cells meet T cells in lymphoid organs

T cells provide help to B cells

Development of plasma cells

Plasma cells home to bone marrow

Antibody enters the bloodstream and reaches the graft

T cell & monocyte infiltrate & tubulitis (invasion of the epithelium)

Antibody mediated rejection

Microcirculation inflammation, C4d staining

T cell mediated rejection

Effector T cells home to graft

T cell activation

T cell mediated rejection

Antibody mediated rejection
THE SIGNIFICANCE OF THE ANTI–CLASS I ANTIBODY RESPONSE

I. CLINICAL AND PATHOLOGIC FEATURES OF ANTI–CLASS I–MEDIATED REJECTION

PHILIP F. HALLOREN,2,3 ARTURO WADGYMAR, SUSAN RITCHIE, JUDY FALK, KIM SOLEZ,2 AND NANGALI S. SRINIVASA2

University of Alberta Hospitals, Edmonton, Alberta; and Toronto General Hospital, Toronto, Ontario, Canada

In renal transplantation, preformed cytotoxic antibody against donor HLA class I antigens causes hyperacute rejection of renal allografts, but its pathogenic significance when it develops in the posttransplant period is unknown. In the present studies we describe the clinical and pathologic features of patients with rejection associated with anti–class I. In the course of 400 consecutive cadaveric renal transplants, 7 patients were identified who had antibody against donor class I HLA antigens in association with atypical but distinctive patterns of rejection. All 7 were presensitized. In 3 patients, the transplant had been inadvertently performed with a positive donor-specific T cell crossmatch. In the remaining 4, the T cell crossmatch on current sera was negative but became positive posttransplant. The clinical picture was deterioration of graft function with rapid onset of oliguria, apparently due to acute tubular necrosis, but with persistence of blood flow demonstrable by radioisotope scan studies. Renal histology showed that the typical lesions observed in cell-mediated rejection, such as tubulitis and interstitial infiltration, were absent. Granular complement deposition (2), polynuclear infiltrates were absent (2) or not prominent (4). In 3 patients the glomerular changes resembled a picture of hemolytic uremic syndrome, with capillary fibrin thrombi and widening of the subendothelial space. IgG staining was negative. The pathologic features suggest that anti–class I antibody appearing or persisting in the early posttransplant period injures the endothelium of the microvasculature, with the clinical presentation different from that of hyperacute rejection. Particularly in sensitized patients, rapid deterioration in function, leading to a picture of acute tubular necrosis, with pathologic features of endothelial injury in the microcirculation, should suggest the diagnosis of anti–class I–mediated rejection.

In renal transplantation the classic consequence of preformed antibody against HLA class I antigens of the donor is hyperacute rejection. The hallmarks of hyperacute rejection include early onset within minutes or hours, cessation of function, and rapid progression to nonviability due to vascular destruction.
## ABMR vs. TCMR

<table>
<thead>
<tr>
<th></th>
<th>No clinical Rejection</th>
<th>ABMR</th>
<th>TCMR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>132</td>
<td>15</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Endothelial transcripts</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>.001</td>
</tr>
<tr>
<td>Ifng dependent transcripts</td>
<td>1.5 ± 0.4</td>
<td>2.1 ± 0.4</td>
<td>2.4 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Macrophage associated transcripts</td>
<td>1.4 ± 0.3</td>
<td>2.1 ± 0.4</td>
<td>2.4 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>CTL associated transcripts</td>
<td>1.2 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>2.0 ± 0.6</td>
<td>.01</td>
</tr>
</tbody>
</table>

*Banu Sis et al AJT (in press 2009)*

Independent-samples T test, ABMR vs. TCMR
• The transcriptome project
• Diagnoses are based on non specific features
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The molecules that predict failure are those associated with response to injury, matrix remodelling, TGFβ effects.

Inflammation does not strongly predict failure.
New definition of kidney ABMR:
(234 kidney biopsies for cause)

- DSA plus microcirculation inflammation
- C4d is less important than microcirculation changes (inflammation, deterioration)
- ABMR is responsible for most late kidney transplant (death censored) loss
- Main disease is de novo anti class II or class II+I, presenting > 1 year, targeting the microcirculation
- TCMR plays little role in kidney loss
- Hyalinosis, scarring are not major factors
• The transcriptome project
• Diagnoses are based on non specific features
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• **Summary**
Summary: lessons from transcripts

• Transcripts provide lessons for diagnostics
  – guide new classifications, new assays
• Diagnoses are based on non specific features
• Changes in diseased tissues are stereotyped
  – interpret single molecule changes in that context
• Canonical T cell mediated rejection
  – about 50% of cases may be non specific
• Antibody mediated rejection goes beyond C4d
  – nearly 2/3 of all late kidneys lost
• Injury and matrix remodelling predict progression
Thank you
and congratulations