Post-Transplant Monitoring in Solid Organ Transplant and HSC Transplant

Marilyn Pollack
Professor, Pathology Dept., Univ. TX HSC San Antonio
President, Am. Soc. Histocompatibility & Immunogenetics
Post Transplant Monitoring

For Solid Organ Transplantation –
1. **Donor Specific Antibody Identification** to help rule in/rule out or predict antibody-mediated Rejection
2. Immune Function testing to adjust immunosuppressive medications or to assess donor specific tolerance
3. Chimerism identification – organ derived graft vs. host dis.

For Allogeneic (!) Hematopoietic Stem Cell Transplantation –
1. Donor Specific Antibody Identification for mismatches (to explain engraftment failure)
2. Chimerism identification to monitor engraftment
3. Identification of Non-HLA polymorphismss affecting GvHD
NEW Type(s) of Organ Transplants – Vascularized Composite Tissue –
Will likely require Different Post – Transplant Monitoring Protocols

A few years ago, a team of military and civilian surgeons transplanted a new hand onto a retired Air Force master sergeant who lost hers nine years ago when a package bomb exploded at Lackland AFB, TX. Starting in 7/2014 such grafts will be regulated by UNOS/OPTN
Flow Cytometry Crossmatching – could be used to identify DSA post-transplant but would require surrogate cells*

*Currently, labs rarely store viable donor cells – high cost for liquid nitrogen
Instead, Microarray Assays with Donor Specific Beads or Panels are Used
Post-Organ Transplant Monitoring

Donor Specific Antibody (DSA) Identification to help rule in or rule out (or predict) Antibody-mediated Rejection (AMR) and to monitor the effectiveness of treatment for AMR

- Post-transplant Crossmatch Tests usually not possible (viable donor cells required – could use surrogate cells but difficult
- Identification of DSA is highly correlated with C4d Staining
- Correlations are with HLA-A,B and/or DR antibodies
- The role of HLA-C, DQ and/or DP antibodies is controversial
- The absence of DSA could actually be due to absorption in the graft

Immune Function Tests to consider adjustment of immunosuppressive meds or assess donor tolerance

- Blood drug levels don’t accurately predict drug effects
- Lymphocyte counts don’t predict lymphocyte function
- Some IS withdrawal protocols require DS tolerance

Chimerism Tests can Help Diagnosis Organ Derived GvHD
Example of Class I Microarray Antibody Identification Results
Immune Function and Other Traditional **Cellular** Assays in Transplantation and Immunogenetics

- **Mixed Leukocyte Culture (MLC)/ Primed Lymphocyte Typing**-
  - Assessment of HLA Class II compatibility measured as uptake of $^3$H thymidine after 5 days (MLC) or 2 days (primed) co-incubation - CAN ASSESS THE DEVELOPMENT OF DONOR SPECIFIC TOLERANCE

- **Cytotoxic Lymphocytes (CTL)**
  Assessment of immune reactivity as cytotoxic responses of stimulated responder cells against labeled target cells - measured as release of $^{51}$Chromium.

- **Donor Specific Precursor Frequency**
  Limiting dilution assays for MLC, PLT or CT

- **General Immune Function - Conventional**
  The capacity of subject lymphocytes to **proliferate** in response to individual mitogens, antigens, and/or pooled allogeneic cells ($^3$H thymidine–3 days)

- **General Immune Function “Cylex®”**
  - Response to PHA measured in 16 hours
  - Measured as ATP formation (early response measure).
Cell Culture for MLC (or CML) Tests –

MLC test

$\overset{3}{H}-\text{TdR}$

Cell incorporating $\overset{3}{H}-\text{TdR}$

4-6 h

MLC assay

Culture medium containing $^{51}\text{Cr}$

4-5 d

$\overset{^0}{A}$ responding cells (effector)

$\overset{^0}{B}_{\text{ss}}$ stimulating cells (sensitising)

$\overset{^0}{B}$ target cells ($^{51}\text{Cr}$ labelled)

F. H. Bach, M. L. Bach, and P. M. Sondel
Time course kinetics of a primary MLC response. Incorporation of tritiated thymidine by proliferating cells usually reaches a maximum at day 6-7.
# IMMUNE RESPONSE TEST REPORT

**Test Date(s):** 1/10/07-1/16/07  
**Technologist(s):** KMR/BCH/LLS  
**Sample Date:** 1/9/07  
**Specimen #:** W00827708  
**Patient:** MC  
**Report Date:** 1/17/07  
**Physician:** Dr. Atkins  
**Hospital:** SWTMH

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Control #1</th>
<th>Control #2</th>
<th>Control #3</th>
<th>Mean of</th>
<th>Controls</th>
<th>RPI***</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PHA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 ug/ml</td>
<td>245,246</td>
<td>217,170</td>
<td>247,665</td>
<td>186,283</td>
<td>217,039</td>
<td>1.13</td>
<td></td>
</tr>
<tr>
<td>10 ug/ml</td>
<td>220,431</td>
<td>210,875</td>
<td>230,728</td>
<td>153,740</td>
<td>198,447</td>
<td>1.11</td>
<td></td>
</tr>
<tr>
<td>5 ug/ml</td>
<td>161,047</td>
<td>177,240</td>
<td>206,555</td>
<td>149,203</td>
<td>177,666</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>2.5 ug/ml</td>
<td>56,856</td>
<td>120,584</td>
<td>112,211</td>
<td>101,972</td>
<td>111,589</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td><strong>CON A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 ug/ml</td>
<td>125,153</td>
<td>93,687</td>
<td>152,108</td>
<td>158,631</td>
<td>134,809</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>10 ug/ml</td>
<td>67,839</td>
<td>60,249</td>
<td>110,365</td>
<td>135,234</td>
<td>101,949</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>5 ug/ml</td>
<td>55,882</td>
<td>50,826</td>
<td>102,900</td>
<td>130,393</td>
<td>94,706</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>2.5 ug/ml</td>
<td>32,588</td>
<td>30,845</td>
<td>77,929</td>
<td>110,125</td>
<td>72,966</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td><strong>PWM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:100</td>
<td>45,221</td>
<td>33,889</td>
<td>36,431</td>
<td>105,003</td>
<td>58,441</td>
<td>0.77</td>
<td></td>
</tr>
<tr>
<td>1:500</td>
<td>73,146</td>
<td>37,831</td>
<td>59,405</td>
<td>120,294</td>
<td>75,510</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>1:1500</td>
<td>79,185</td>
<td>48,722</td>
<td>74,714</td>
<td>131,792</td>
<td>85,283</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>1:4500</td>
<td>98,152</td>
<td>57,097</td>
<td>88,228</td>
<td>154,616</td>
<td>99,981</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td><strong>Pooled Allogeneic Cells Test 1</strong></td>
<td>134,295</td>
<td>94,234</td>
<td>139,916</td>
<td>163,398</td>
<td>136,223</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td><strong>Pooled Allogeneic Cells Test 2</strong></td>
<td>122,185</td>
<td>116,004</td>
<td>151,037</td>
<td>165,657</td>
<td>95,887</td>
<td>1.30</td>
<td></td>
</tr>
</tbody>
</table>
Cylex® Test for “General” Immune Function

Lymphocyte Stimulation w/ PHA

ATP detection reagents

Cell lysis to release ATP

Magnetic separation of CD4 cells.

ATP

Incubate 15-18 hrs.

Wash

Measure light intensity

ATP + luciferin $\xrightarrow{\text{Luciferase}}$ Oxyluciferin + AMP + PPI + light

$\text{Mg}^{2+} + O_2$

$\text{hv (562 nm)}$

The intensity of the light emitted is directly proportional to the amount of ATP in the sample. Comparison to ATP standards allows quantifiable measurement of ATP produced by activated cells.

An ATP Standard Curve is Used to Calculate Results for Patient Tests
Cylex Responses in “Stable” Transplant Recipients

From Kowalski et al, Transplantation, 2006
Cylex Responses of Solid Organ Transplant Recipients During Periods of Confirmed Rejection or Documented Infection

From Kowalski et al, Transplantation, 2006
THE USE OF POLYMORPHIC PCR-STR (and -VNTR) SYSTEMS TO TEST FOR CHIMERISM AFTER ORGAN TRANSPLANTATION

• Some Tolerance Induction Protocols infuse organ donor bone marrow or lymphocytes to try to create tolerance/donor-specific suppressor cells
• For those protocols, systems are needed to monitor chimerism
• Organ Donor-Derived Lymphocytes can cause Graft vs. Host Disease, especially after Liver or Small Bowel Transplantation
  • Grafts contain large numbers of donor lymphocytes
  • Immunosuppression can prevent rejection of donor lymphocytes
PCR Reaction for (STR) Short Tandem Repeats

The repeat region is variable between samples while the flanking regions where PCR primers bind are constant.

Homozygote = both alleles are the same length
Heterozygote = alleles differ and can be resolved from one another

In multiplex systems, different primers can use different dyes, but the same dyes can be used when products are different sizes.
Commonly used STR Marker Locations

(13 CODIS (Core STR) Loci Used by the FBI)
Pollack MS, et al., Severe, Late-onset Graft-Versus-Host Disease in a Liver Transplant Recipient Documented by Chimerism Analysis. Human Immunology. 2005
Example of typical STR multiplex analysis results:

- Note somewhat smaller areas of larger bands in most systems
  - Row 1 = xy + known alleles for 3 different STR systems
  - Row 2/3 = recipient/donor pre-transplant
  - Row 4 = post-transplant sample – 2 informative systems without interfering stutter bands. (The last system would not have been usable if the 2nd recipient band had been 1 allele larger - interfering stutter band)
HLA haplotypes for a case report family: Monitoring Antibody mediated rejection to evaluate cause and treatment for HPC transplant*

Patient

- a A*32, B*35, Cw*04, DRB1*11, DRB3
- d/c A*29/, B*07, Cw*07, DRB1*01

Father:

- a A*32, B*35, Cw*04, DRB1*11, DRB3
- b A*24, B*38, Cw*NT, DRB1*14, DRB3

Mother:

- c A*68, B*07, Cw*07, DRB1*01
- d A*29, B*45, Cw*NT, DRB1*12, DRB3

Brother#1: (donor)

- a A*32, B*35, Cw*04, DRB1*11, DRB3
- c A*68, B*07, Cw*07, DRB1*01

Brother#2:

- b A*24, B*38, Cw*NT, DRB1*14, DRB3
- c A*68, B*07, Cw*07, DRB1*01

Sister:

- a A*32, B*35, Cw*04, DRB1*11, DRB3
- d A*29, B*45, Cw*NT, DRB1*12, DRB3

*Pollack, MS and Ririe D. Clinical significance of recipient antibodies to stem cell donor mismatched class I HLA antigens. Human Immunology 2004