Organ transplantation remains the gold standard treatment strategy for individuals with end-stage organ failure. Despite improved immunosuppressive regimens, rejection remains a significant problem that limits long-term survival of allografts. Responses to alloimmune human leukocyte antigen (HLA) molecules are critical for the development of rejection, but the changes in T-cell alloreactivity that contribute to this process are not fully understood. T-Cell receptor (TCR) repertoire analysis is a powerful tool for assessing changes in the development of rejection, but it is 1) at least 20,000 unique TCR sequences. We first established a method for alloreactive T-cell repertoire analysis using a pre-transplant mixed lymphocyte reaction and high throughput sequence platform in 2014. Since then, similar technologies have been used to investigate mechanisms of rejection and tolerance in small cohorts. 2,4

To operatively identify donor reactive T-cell clones (DRTC) in a cohort of kidney transplant recipients

To monitor the presence of DRTC in the post-transplant period to identify signatures of allograft rejection

**Experimental Methods**

**A)** Pretreatment Donor-specific MLR (TCR) Identification

B) Flow sorting of CD4- and CD8- DRTC

C) Adaptive Immunosequencing

D) PBMC from Transplant

E) Flow cytometry

**Figure 1:** Methodology to identify donor reactive T-cell clones (DRTC). A) CFSE-labeled recipient responder cells were stimulated with PHA-20 stimulated, irradiated donor stimulators. B) After 7 days, the CFSE-labeled responder cells were flow sorted into their CD4- and CD8- subfractions. C) Flow sorted populations were then subjected to the Adaptive Immunosequencing platform by Adaptive Biotechnologies to simultaneously sequence all TCR beta-chain sequences. D) CD4- and CD8- DRTC were then identified by plotting the TCR beta-chains reads from the MLR sorted CD4- and CD8- subfractions against the unstimulated pre-transplant PBMC. E) Circulating CD8+ DRTC were increased in subjects that received non-identical donors. The number of CD8+ DRTC was strongly correlated with their frequency (r^2=0.05, p=0.37). F) Baseline (i.e., pre-transplant) CD4- and CD8- DRTC number and frequency were evaluated to determine if there was a difference in subjects that ultimately develop rejection/borderline rejection. No significant difference was observed with CD4- DRTC, but both the absolute number and frequency of CD8- DRTC were increased in those subjects (Mann Whitney U Test).

**Figure 2:** Generation and characterization of DRTC in the pre-transplant period. DRTC were identified as described in Figure 1. A) A median of 930 CD4- and 113 CD8- DRTC were detected. B) The number of DRTC were then compared across the number of HLA-matches based on the following loci: HLA-A, HLA-B, HLA-C, and HLA-DR. Increased generation of DRTC was observed in subjects with greater HLA disparity (Walls Test, CD4). The frequency of CD4- and CD8- DRTC was then plotted against the absolute number of each DRTC subset. The number of CD8- DRTC was strongly correlated with their frequency (r^2=0.05, p=0.37) which was not observed with CD4- DRTC (p=0.05, q=0.01). C) Baseline (i.e., pre-transplant) CD4- and CD8- DRTC number and frequency were evaluated to determine if there was a difference in subjects that ultimately develop rejection/borderline rejection. No significant difference was observed with CD4- DRTC, but both the absolute number and frequency of CD8- DRTC were elevated in non-matched subjects (Mann Whitney U Test).

**Cohort Characteristics**

Table 1: Characteristics of subjects that had a normal (Stable) vs abnormal (Non-Stable) biopsy in the post-transplant period

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Male</th>
<th>White</th>
<th>HLA Class I</th>
<th>HLA Class II</th>
<th>Body Mass Index (kg/m²)</th>
<th>Pre-operative Day 1 Lymphocytes (%)</th>
<th>Pre-operative Day 1 NK Cells (%)</th>
<th>Pre-operative Day 1 Beta Chain Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilized</td>
<td>35.8±1.0</td>
<td>91%</td>
<td>90%</td>
<td>90%</td>
<td>22.1±4.9</td>
<td>40.7±4.9</td>
<td>1.0±0.5</td>
<td>0.01%</td>
</tr>
<tr>
<td>Non-Stable</td>
<td>36.0±1.0</td>
<td>87%</td>
<td>91%</td>
<td>91%</td>
<td>22.1±4.9</td>
<td>40.7±4.9</td>
<td>1.0±0.5</td>
<td>0.01%</td>
</tr>
</tbody>
</table>

**Conclusions**

Increased pre- and post-transplant, circulating CD8+ DRTC are associated with development of rejection in subjects that receive non-identical transplantation.

The majority of CD8+ DRTC detected in the allograft at rejection can be readily identified in the pre-transplant PBMC.

In-depth characterization of pre-transplant DRTC may enable risk stratification of subjects in the early post-transplant period.

**References**


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