Serial monitoring of the alloreactive T-cell repertoire in kidney transplant recipients receiving non-lymphodepletional induction

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Introduction

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 allogeneic 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 repertoires over time given its ability to simultaneously identify thousands of unique TCR sequences. We first established a methodology for alloreactive T-cell repertoire analysis using a pre-transplant mixed lymphocyte reaction and high throughput sequence platform in 2014[1]. Since that time, similar technologies have been used to investigate mechanisms of rejection and tolerance in small cohorts[2-5].

Research Objectives

- ❖ *To pre-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

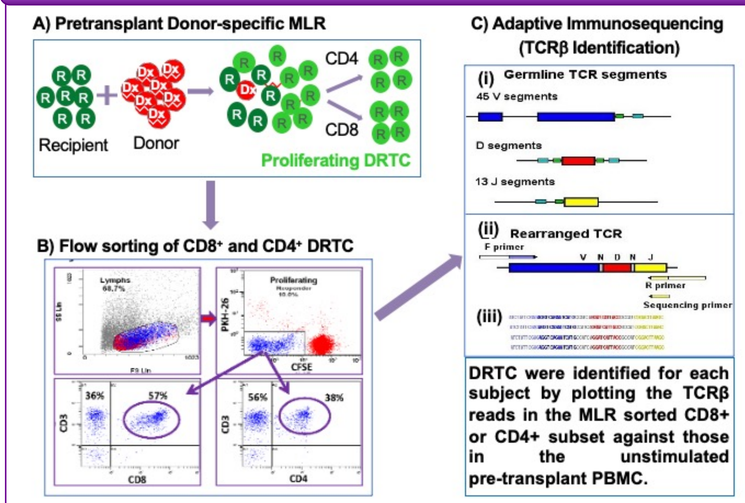
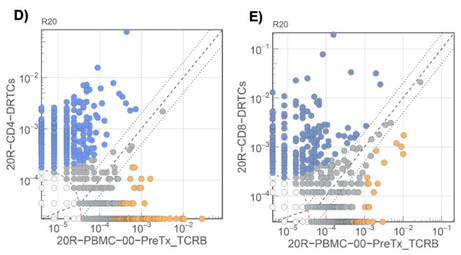


Figure 1: Methodology to identify donor reactive T-cell clones (DRTC). A) CSFE-labeled recipient responder cells were stimulated with PKH-26 labeled, irradiated donor stimulators. **B)** After 7 days, the CSFE-diluting responder cells were flow sorted into their CD4+ and CD8+ subsets. **C)** Flow sorted populations were then subjected to the Adaptive Immunosequencing platform by Adaptive Biotechnologies to simultaneously sequence all TCR-beta chain sequences. **D)** CD4+ and **E)** CD8+ DRTC were then identified by plotting the TCR-beta chain reads from the MLR flow sorted CD4 and CD8 samples against the unstimulated, pre-transplant PBMC. DRTC (blue circles) were clones that were: 1) significantly more abundant in the MLR sort based on a binomial model after multiple comparisons corrections and 2) at least 2-fold more abundant in the MLR sort compared to the unstimulated pre-transplant sample.



Cohort Characteristics

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

	Stable (3 mo) (N=11)	Non-Stable (3 mo) (N=9)	p-value
Age (years)*	56.8 ± 13.6	51.1 ± 17.0	0.41
Race; N (%)			
White	10 (90.9%)	6 (66.7%)	0.28
Other	1 (9.1%)	3 (33.3%)	0.28
Male; N (%)**	6 (54.5%)	5 (55.6%)	>0.99
Body-Mass Index (kg/m2)	32.3 ± 4.9	30.5 ± 4.2	0.39
Prior Transplant; N (%)	1 (9.1%)	1 (11.1%)	>0.99
Pre-operative Dialysis; N (%)	4 (36.7%)	4 (44.4%)	>0.99
Deceased Donor; N (%)	0 (0%)	2 (22.2%)	0.19
Donor Age (years)	44.1 ± 15.8	46.9 ± 13.7	0.68
Male Donor; N (%)	7 (63.6%)	5 (55.6%)	>0.99
Pre-operative DSA; N (%)	2 (18.2%)	0 (0%)	0.48
ABO Incompatible; N (%)	1 (9.1%)	0 (0%)	>0.99
Induction Regimen; N (%)			
Simulect	9 (81.8%)	9 (100%)	0.48
Solumedrol	2 (18.2%)	0 (0%)	0.48
Additional Pre-operative Regimen; N (%)			
Rituxan	3 (27.3%)	0 (0%)	0.22
TPE/IVIG	1 (9.1%)	0 (0%)	0.22
Documented DGF; N (%)	1 (9.1%)	2 (22.2%)	0.57
Dialysis at 12 months; N (%)	0 (0%)	0 (0%)	>0.99
Graft failure; N (%)	0 (0%)	0 (0%)	>0.99
HLA Mismatch >3/6***	3 (27.3%)	8 (88.9%)	0.01

*Continuous variables are reported as mean ± SD and were compared using Student's unpaired T-test
**Categorical variables are reported as N (%) and were compared using Chi-squared or Fischer's Exact Test
***HLA mismatch was evaluated at the following loci: HLA-A, HLA-B, and HLA-DR

Pre-Transplant Characterization of DRTC

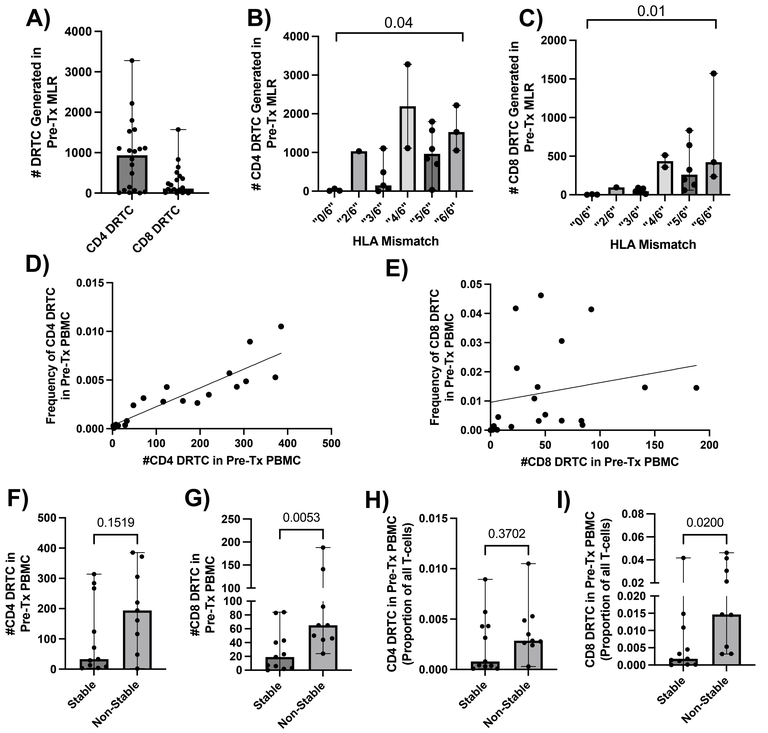


Figure 2: Generation and characterization of DRTC in the pre-transplant period. DRTC were identified as shown in Figure 1. **A)** A median of 935 CD4+ and 113 CD8+ DRTC were detected. **B-C)** The number of DRTC were then compared across the number of HLA mismatches based on the following loci: HLA-A, HLA-B, HLA-DR. Increased generation of DRTC was observed in subjects with greater HLA disparity (Kruskal Wallis Test). **D-E)** The frequency of **D)** CD4+ and **E)** CD8+ DRTC was then plotted against the absolute number of each DRTC subset. The number of CD4+ DRTC was strongly correlated with their frequency (r^2=0.79, p<0.0001), which was not observed with CD8+ DRTC (r^2=0.05, p=0.37). **F-I)** Baseline (i.e., pre-transplant) CD4+ and CD8+ DRTC number and frequency were evaluated to determine if there was a difference in subjects that would 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-stable subjects (Mann Whitney U Test).

Circulating CD8+ DRTC Are Increased at Rejection

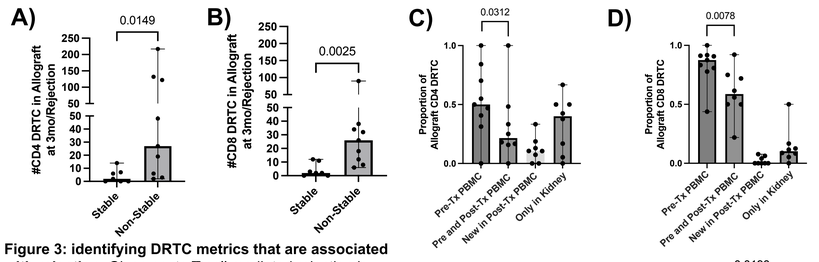


Figure 3: identifying DRTC metrics that are associated with rejection. Given acute T-cell mediated rejection is associated with allograft infiltration by alloreactive T-cells, we first detected DRTC in normal and rejecting biopsy samples. **A-B)** Both CD4+ and CD8+ DRTC were increased in the allograft at rejection (Mann-Whitney U Test). **C-D)** Next, we sought to determine if the DRTC found in the allograft could also be identified in the circulation. The majority of CD8+ DRTC found in the allograft at rejection could be readily identified in the pre-transplant PBMC. Interestingly though, this proportion decreased in the paired PBMC sample suggesting some clones likely track into the allograft and out of the periphery (Wilcoxon Signed Rank Test). Similar trends were observed with CD4+ DRTC, but there was a greater proportion of CD4+ DRTC never detected in the circulation. These clones could be naïve CD4+ T-cell subsets that exist at low frequencies in the periphery but expand upon entry into the allograft. **E-H)** The number of circulating CD4+ and CD8+ DRTC was then compared between stable subjects and those that developed rejection/borderline rejection. As observed in the pre-transplant samples, there was an increased number and frequency of CD8+ DRTC in the circulation of abnormal subjects at rejection. Notably, only the difference in the frequency of CD8+ DRTC was statistically significant (Mann Whitney U Test).

Conclusions

- ❖ Increased pre- and post-transplant, circulating CD8+ DRTC are associated with development of rejection in subjects that receive non-lymphodepletional induction
- ❖ The majority of CD8+ DRTC detected in the allograft at rejection can be detected in the pre-transplant circulating repertoire. These clones may represent higher frequency, memory T-cell subsets
- ❖ In-depth characterization of pre-transplant DRTC may enable risk stratification of subjects in the early post-transplant period

Acknowledgements

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