PIRCHE, DRTC and CTLp Are Directly Correlated with HLA Mismatch and May Be Predictive of Rejection in Kidney Transplant Patients

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Background

- Despite advances in transplant immunology, the complex interactions between HLA mismatch, cellular immune responses and transplant outcomes are not fully understood.
- While HLA compatibility is the cornerstone of pre-transplant risk assessment, the complex interaction between HLA mismatch and the recipient's immune response leads to unpredictable graft survival.
- This complexity highlights critical gaps in predicting transplant rejection risks. Therefore, improved prediction strategies are essential for ensuring sustainable transplant success.

Research Objectives

- Assess the combined predictive power of donor-reactive T cell (**DRTC**) frequency and Predicted Indirectly ReCognizable HLA Epitopes (**PIRCHE**) analysis for acute rejection in kidney transplant recipients.
- Evaluate the correlation between the degree of HLA mismatch and cytotoxic T lymphocyte precursors (**CTLp**) frequencies.

Experimental Schema

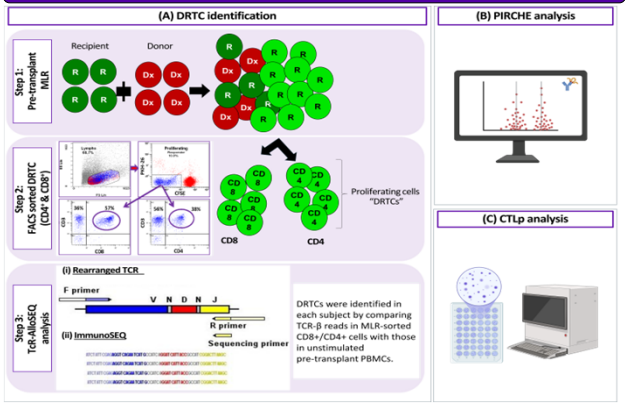

Figure 1. Experimental schema. (A) DRTC identification. CFSE-labeled recipient (R) and PKH-labeled irradiated donor (Dx) PBMCs were co-cultured to generate (R) DRTCs, followed by identification and FACS sorting of CD4+/ CD8+ DRTCs. The TCR-β chains of sorted DRTCs were then amplified and sequenced using TCR-AlloSeq to identify repertoire of TCR sequences. Additionally, **(B) PIRCHE** and **(C) CTLp** analysis were done to determine the extent of immune responses based on the degree of HLA mismatches between (R) and (Dx).

CD4+/ CD8+ DRTC vs. HLA Mismatch

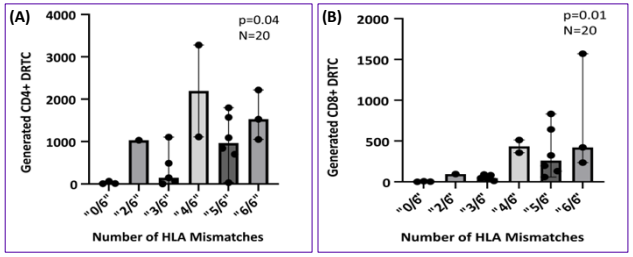

Figure 2. (A) CD4+ DRTC and (B) CD8+ DRTC in relation to number of HLA Mismatches. Generated (A) CD4+ DRTC and (B) CD8+ DRTC were evaluated based on the degree of HLA mismatches in kidney transplant recipients (n=20). HLA loci included two alleles each of HLA-A, HLA-B, and HLA-DR for a maximum 6/6 HLA mismatches. The generation of CD4+/CD8+ DRTCs significantly correlates with the degree of HLA-mismatch between donor and recipient.

PIRCHE-II Correlation with Total DRTC

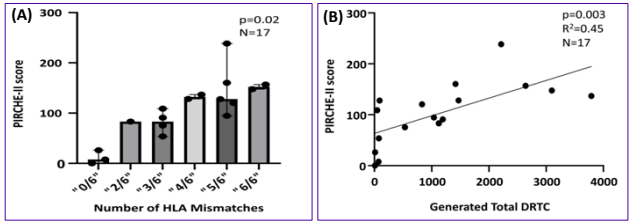

Figure 3. PIRCHE-II Correlation with (A) number of HLA mismatches and (B) Total DRTC. (A) Higher PIRCHE-II scores correlates significantly with a greater number of HLA-mismatches (n=17). (B) PIRCHE-II scores linearly correlated with total DRTC (n=17); data points represent individual patients.

Limitations

- Cohort size. Next step is to expand the cohort to achieve sufficient statistical power and representativeness.
- Lack of mechanistic findings. Incorporate in-depth analysis to investigate the precise molecular and cellular mechanisms driving the observed correlation between generated DRTCs, PIRCHE-II scores, CTLp frequencies with HLA mismatches.

CTLp frequency vs. HLA Mismatch

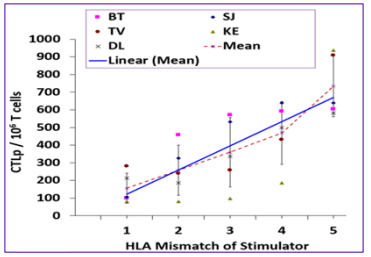

Figure 4. Analysis of Cytotoxic T-Lymphocyte precursor (CTLp) in relation to HLA-mismatches. CTLp frequencies of 5 individual 'normal' laboratory volunteer responders (R) against donor stimulators (Dx) with indicated HLA mismatches were assessed using classical limiting dilution analysis. A significant increase in CTLp frequency was observed with greater number of HLA-mismatches between Recipient and Donor, indicating a heightened alloreactive immune response with greater HLA disparity.

Increased PIRCHE-II scores & CD8+ DRTC in Abnormal Biopsies

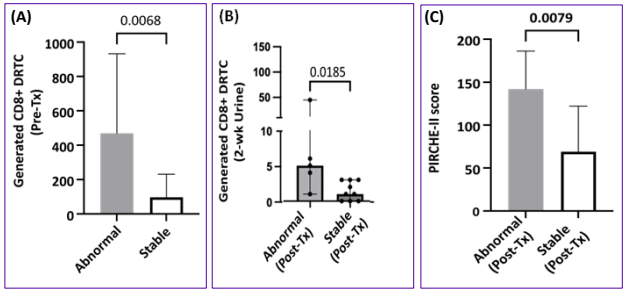

Figure 5. Elevated (A-B) CD8+ DRTC and (C) PIRCHE-II scores is associated with the occurrence of post-transplant (post-tx) abnormal biopsies (Stable=9, Non-stable=5). Increased post-tx CD8+ DRTC and PIRCHE-II scores in abnormal biopsies indicate the presence of a systemic response.

Conclusion

- Our results show a direct association between the degree of HLA mismatch versus generated DRTCs, PIRCHE-II scores and CTL immune responses, with detrimental effects observed post-transplantation.
- This underscores the need for careful and comprehensive immunological evaluations of recipients against donors prior to transplantation.

Disclosure

Jeyamogan S: **None**. Sanders JM: **None**. He J: **None**. Niemann M: **Employee of PIRCHE ® AG, a commercial entity**. Xu H: **None**. Leventhal JR: **None**. Mathew JM: **None**.

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