Identification and function of a CD4⁺/CD8αβ⁺ T cell population that is predictive of GVHD development in a xenogeneic transplant model

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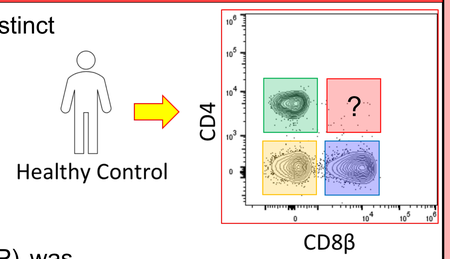
Graft-vs-host disease (GVHD) is mediated by donor reactive T cells that have a hierarchical classification based on CD4 and CD8 expression. While CD4 and CD8 lineages are thought to have fixed expression, CD4⁺/CD8αβ⁺ double positive (DP) T cells have been reported in cases of human cancers and autoimmune diseases though the lack of a suitable model system has hindered their research. In this study, we transplanted primary human graft tissue into non-conditioned immunodeficient mice and observed the development of a human DP T cell population that was not present in the starting grafts. This DP T cell population developed irrespective of graft tissue (peripheral blood, bone marrow or umbilical cord blood), accessory cells (transplantation with isolated T cells) and immunodeficient mouse strain (NSG and NBSGW). Furthermore, an increase in the percentage of DP T cells in the blood of these mice is correlated and predictive of GVHD development. We also observed that DP T cells are functionally active with significantly elevated IFNγ and TNFα secretion compared to CD4 and CD8 single positive T cells. DP T cell also display elements of the cytotoxic machinery including NKG2D and perforin/granzyme expression. Interestingly, transplantation of isolated CD4⁺ cells did not result in the development of DP T cells while a robust population developed after transplantation of isolated CD8⁺ T cells. DP T cells were also identified in primary clinical samples taken from HSCT patients with their clinical relevance to GVHD currently under investigation. In conclusion, this ongoing study has identified a novel human DP T cell that arises from the CD8⁺ T cell population, is functional active and is predictive of GVHD in a xenogeneic transplant model.

Introduction/Key Points

- T cells are generally divided into distinct lineages based on their CD4/CD8 expression
  - CD4+ (adaptive modulatory)
  - CD8+ (adaptive cytotoxic)
  - CD4-/CD8- [DN] (innate-like cytotoxic)
- The presence of CD4+/CD8+ (DP) was thought to be restricted to T cell progenitors developing in the thymus
- Recent studies now suggest that mature DP T cells may develop in the periphery during chronic inflammatory diseases



CD4⁺/CD8⁺ double-positive T cells: more than just a developmental stage?
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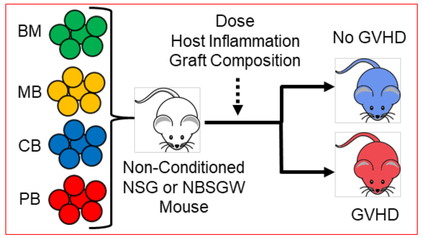
RECEIVED AUGUST 6, 2014; ACCEPTED SEPTEMBER 2, 2014; DOI: 10.1189/jlb.1RU0814-392

Xenogeneic Transplant Model For GVHD Research



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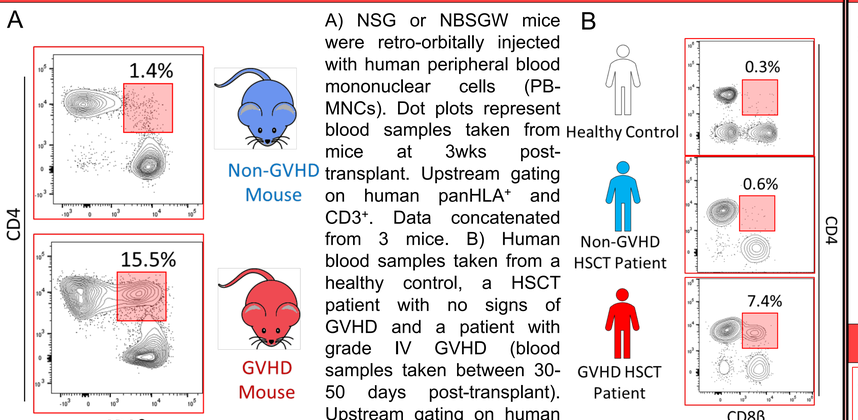
- Human mononuclear cells (or isolated T cells) are retro-orbitally injected into non-conditioned NSG or NBSGW mice
- GVHD development is not inevitable and is dependent on graft source, graft composition, dose and the host inflammatory environment

Working Hypothesis

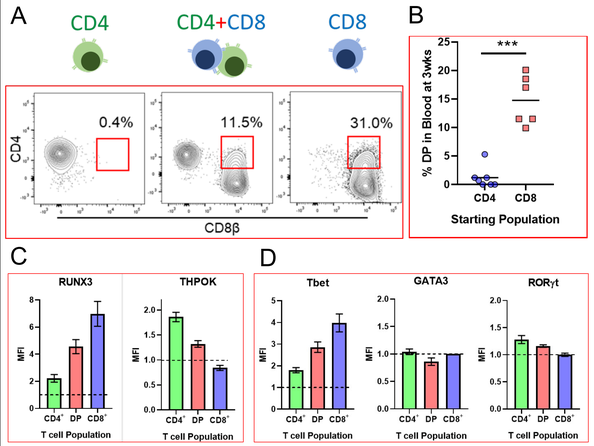
Human CD4+/CD8αβ+ (DP) T cells originate from the CD8+ population and are sufficient to mediate GVHD pathology

CD4+/CD8+ (DP) T cell Develop During GVHD



A) NSG or NBSGW mice were retro-orbitally injected with human peripheral blood mononuclear cells (PB-MNCs). Dot plots represent blood samples taken from mice at 3wks post-transplant. Upstream gating on human panHLA⁺ and CD3⁺. Data concatenated from 3 mice. B) Human blood samples taken from a healthy control, a HSCT patient with no signs of GVHD and a patient with grade IV GVHD (blood samples taken between 30-50 days post-transplant). Upstream gating on human panHLA⁺ and CD3⁺.

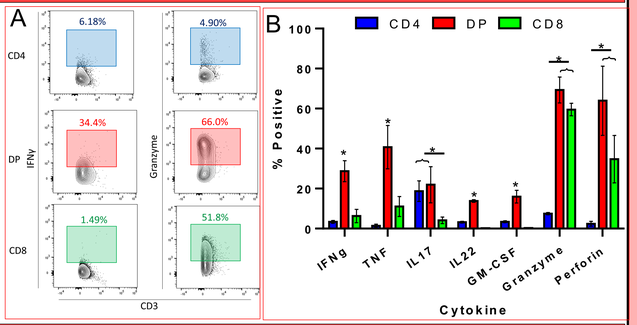
DP T cell Originate From the CD8 Population



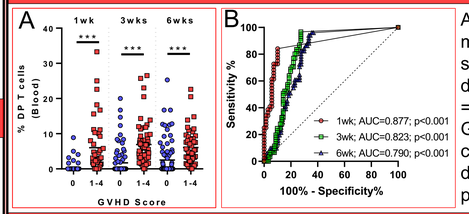
A) Isolated human T cells (CD4+CD8), CD4 or CD8 T cells were transplanted into NSG or NBSGW mice. Dot plots represent blood samples taken at 3wks post-transplant. B) Graphical representation of results from (A). C) Bar graphs showing the median fluorescent intensity over isotype staining for human T cells stained for the CD8 and CD4 master transcription factors RUNX3 and THPOK respectively. D) Human T cells were stained as in (C) for the effector determining transcription factors Tbet (TH_1), GATA3 (TH_2) and RORγt (TH_{17}).

DP T cell Express Both Cytotoxic and Modulatory Cytokines

A) Blood samples taken from mice given PB-MNC were cultured for 6hrs in the presence of brefeldin A before staining for the indicated cytokines. B) Graphical representation of the results from (A). Results are compiled from 5 mice. * p<0.05. Blue = CD4⁺; Red = DP; Green = CD8.



DP T cells Are A Prospective Predictive Biomarker of GVHD



A) The percentage of DP T cells in the blood of mice at 1, 3 and 6wks post-transplant is significantly correlated with lethal GVHD. Each dot represents an individual mouse. Blue circle = 12wk GVHD score of 0, Red Square = 12wk GVHD score of 1-4. B) Receiver-operator-characteristic (ROC) curve was used to determine feasibility of using DP T cells as a predictive biomarker of GVHD. *** p<0.001

Conclusions

- Human CD4⁺/CD8⁺ (DP) T cells are a relevant and functionally active antigen-experienced (CD45RO⁺) population that develops during GVHD.
- DP T cells arise from CD8⁺ T cells, express both RUNX3 and THPOK and secrete cytotoxic (CD8 lineage) and modulatory (CD4 lineage) cytokines.
- The development of DP T cells is predictive of GVHD in our xenogeneic transplant model and we are actively exploring the feasibility of using DP T cells as a predictive biomarker of GVHD in the clinic.

Mentorship & Funding Support

Capitini & Gumperz Laboratories; UW-Madison Transplantation T32 (T32-AI125231); Hematology T32 (T32-HL07899); Cormac Pediatric Leukemia Fellowship; SCRMC Post-Doctoral Fellowship; ICTR Translational Pilot Award

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