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+CD8β+ 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 observed 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+ T 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

**Xenogeneic Transplant Model For GVHD Research**

- Human mononuclear (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

**DP T cell Originate From the CD8 Population**

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

**Working Hypothesis**

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

**Conclusion**

- 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.

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