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POSTER ABSTRACTS

701.EXPERIMENTAL TRANSPLANTATION: BASIC AND TRANSLATIONAL

Testing the Limits of Endogenous Thymic Regeneration after HCT Conditioning

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T cell reconstitution following allogeneic hematopoietic cell transplantation (allo-HCT), recently emerging as a major predictor of clinical outcomes, is critically dependent upon thymic function. Previous clinical studies have implicated a link between thymic function pre-transplant to patient outcomes post-bone marrow transplantation. Thymic function is primarily driven by two functionally distinct groups of thymic epithelial cells (TECs) located in the cortex (cTECs) and the medulla (mTECs). These two stromal compartments contribute to the various stages of T cell development, culminating in the generation of a broad repertoire of self-tolerant T cells leaving the thymus and emigrating into the periphery. However, despite the thymus' crucial role in generating a broad array of T cells, the organ is highly sensitive to damage. Condition regiments required for successful HCT, such as ionizing radiation, have a profound damaging effect on the thymus. Although the thymus is sensitive to damaging agents, it also has a remarkable self-repair capacity through mechanisms that are still incompletely understood; and the organ's regenerative capacity also declines with age. Moreover, the specific thymic epithelial subpopulation that contributes to thymus regeneration remains undefined. Therefore, understanding endogenous cellular mechanisms of thymus regeneration, and limitations to its repair, will be crucial for creating therapies to boost thymic function.

Although thymic function was restored to pre-conditioning levels after one round of total body irradiation (TBI), and multiple rounds of corticosteroid (Dexamethasone) damage saw no decline in its restorative ability, thymic regeneration was severely reduced upon subsequent rounds of ionizing radiation damage (Fig. 1A). A phenomenon that one may expect to see in the small subset of patients that need to undergo a second HCT. Using single cell RNA sequencing of highly purified TECs at multiple consecutive days after TBI, we identified that shortly after damage the TEC compartment contracts into distinct TEC subpopulations, including a previously reported CD104 expressing mTEC1 subpopulation implicated to provide pre-cursor support to the thymic medulla (Borstein 2018 *Nature*). To further understand the fundamental differences in regeneration from these different damage modalities, we compared the regeneration of thymic epithelial cells after either dexamethasone (Dex) or TBI damage. Consistent with its progenitor role, we found a significant decrease in the mTEC1 subpopulation in mice damaged with TBI compared to those damaged with Dex. The striking distinction in the mTEC1 compartments between the damage models can potentially explain a key functional difference in how these two damage modalities differ in their regenerative kinetics (Fig 1B).

Overall, the results of these studies implicate a potential contribution of a specific mTEC subset (mTEC1s) to modulating thymic regeneration kinetics, and that the thymus cannot withstand multiple rounds of irradiation. The identification of mTEC1s as crucial mediators of thymic regeneration can inform future work in designing thymic boosting therapies that induce this stromal population to accelerate thymus regeneration after HCT.

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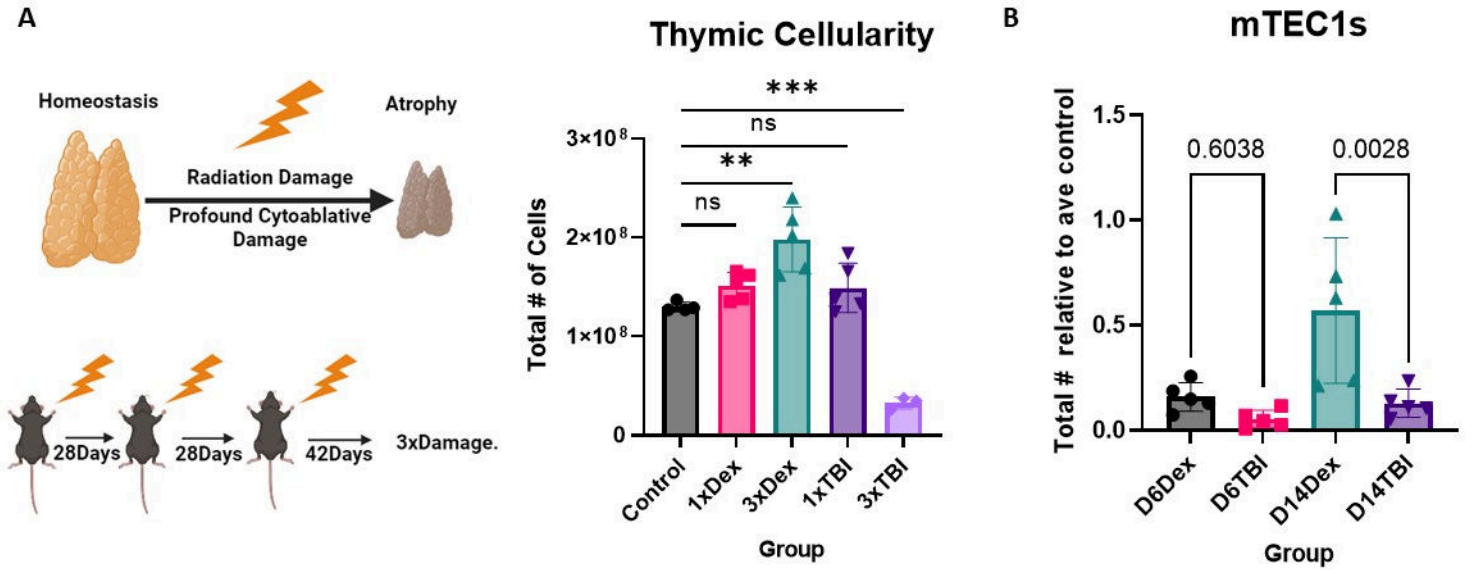

Figure 1: A, 8-10-week-old female C57BL5/j mice were either damaged with 20mg/kg of Dex or 550cGY of TBI. Mice were damaged multiple times at 28-day intervals and were harvested 42-days after the last damage induction. N=5 per group and P-values imply difference against undamaged controls using an unpaired t-test. **B,** similar design as in **A**, singly damaged mice were taken down at the indicated timepoints. Flow cytometry analysis was pregated on CD45- Ter119- EpCAM+ MHCII+. Absolute numbers of mTEC1 (MHCII low, CD104+) cell populations were normalized according to the average of undamaged controls. N=5 per group and P-values generated from a one-way ANOVA.

Figure 1