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POSTER ABSTRACTS

701.EXPERIMENTAL TRANSPLANTATION: BASIC AND TRANSLATIONAL

A Novel High-Throughput Method for Identifying T-Cell Receptor: Minor Histocompatibility Antigen Interactions in Mouse Models of Graft-Vs-Host Disease

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Graft-vs-host disease is a complication of allogeneic stem cell transplant (alloSCT) in which donor T cells target alloantigens expressed by healthy recipient tissues. In major-histocompatibility-matched alloSCT, CD8-mediated GVHD is caused by T cell receptor (TCR) allorecognition of class-I presented minor histocompatibility antigens (miHAs). The vast majority of miHAs remain unknown between any given donor-recipient pairing. However, determining the identities of these miHAs and their cognate TCRs would prove useful towards the development of allografts that minimize GVHD yet preserve the therapeutic benefits of alloSCT. Here, we implement a high-throughput TCR cloning method, TCXpress, to screen alloreactive TCRs from a mouse model of MHC-matched alloSCT against putative miHA targets determined informatically.

129 (H-2 ^{b}) mice, which express the immunodominant miHA H60, were lethally irradiated and reconstituted with bone marrow and CD4 $^{+}$ and CD8 $^{+}$ T cells from B6 (H-2 ^{b}) donors. At day +10, recipient spleens were harvested and CD44 $^{+}$PD-1 $^{+}$ donor CD8 $^{+}$ cells were single-cell sorted according to H-2Kb:H60 tetramer (Tet ^{H60}) staining into unknown miHA-reactive (Tet $^{H60-}$) and control H60-reactive (Tet $^{H60+}$) cohorts for TCR cloning using the TCXpress platform (BlueSphere Bio). Clonotyping was performed by sequencing the CDR3 region of TCRs. Putative miHAs were identified informatically by filtering non-synonymous variants between B6 and 129 reference exomes on predicted class I binding affinities. To interrogate TCR target specificity, Jurkat reporter cells, which express GFP and CD69 upon TCR activation, were transduced with selected alloreactive TCRs and reacted in a multiplexed fashion against a panel of B6WT3 antigen-presenting cells (H-2 ^{b}) transduced with tandem minigenes (TMG) of predicted miHAs. To test TCR reactivity against hematopoietically-expressed miHAs independent of our calling strategy, Jurkat reporters were also reacted against bone-marrow derived dendritic cells (BMDCs) from control B6 and 129 mice.

From 3 recipient mice, 966 alloreactive TCRs were cloned and sequenced (735 Tet $^{H60-}$ and 231 Tet $^{H60+}$) corresponding to 553 unique clonotypes (437 Tet $^{H60-}$ and 116 Tet $^{H60+}$). Sixty-seven percent (N=668) of TCRs sequenced had clonotype occurrences of $\geq$ 2, indicating clonal expansion post-alloSCT, whereas there were no repeated clonotypes among donor B6 CD8 $^{+}$ cells pre-transplant (N=173 TCRs). A total of 47 Tet $^{H60-}$ TCR clones and 3 Tet $^{H60+}$ control TCR clones were selected for transduction into Jurkat reporters and screened against a limited TMG library of 158 putative B6→129 miHAs, as well as H60. Expectedly, all TCR clonotypes isolated from the Tet $^{H60+}$ sort gate reacted against H60-expressing B6WT3 cells and 129 BMDCs. From the Tet $^{H60-}$ TCRs, one clone was validated against another documented 129 miHA, H4, while an additional 4 clones were found to react against novel B6→129 miHAs (Serpinb8 and Cdt1). Interestingly, 2 highly over-represented clones were autoreactive against B6 tissues (B6WT3 cells and BMDCs), but not 129 BMDCs.

In summary, we demonstrate the capacity of a novel TCR cloning platform to validate informatically-predicted miHAs and their cognate TCRs in a mouse model of GVHD. This approach may be readily adapted to develop new TCR:miHA therapeutics and gain a deeper understanding of GVH responses targeting miHAs.

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