Characterization of CMV induced alpha/beta and gamma/delta T cells after allogeneic stem-cell transplantation utilizing single cell RNA seq

Anniek Stuut[1], Anke Janssen[2], Peter Brazda[1], Froso Karaiskaki[1], Trudy Straetemans[1], Dennis Beringer[1], Zsolt Sebestyen[1], Julia Drylewicz[1], Moniek de Witte[2], Jürgen Kuball[1,2]

1.Center for Translational Immunology, University Medical Center Utrecht, The Netherlands
2.Department of Hematology, University Medical Center Utrecht, The Netherlands

Background: Allogeneic stem cell transplantation (allo-SCT) remains the only curative option for various high risk hematological malignancies. To reduce the risk of graft versus host disease, we perform *ex vivo* αβT cell depletion before infusion, which is expected to result in an increased risk of viral reactivations such as cytomegalovirus (CMV). We and others have previously shown that after αβT cell depleted allo-SCT, early immune reconstitution and viral immunity relies predominantly on γδT and NK cells. Here we study how CMV contributes to the development of the innate and adaptive immune repertoires after T cell deplete and T cell replete allo-SCT.
Methods: Numerical reconstitution of αβT and γδT cells was analyzed in a retrospective cohort of patients who had received an αβT cell depleted allo-SCT (n=146) or T cell replete allo-SCT (n=67). For 30 αβT cell depleted allo-SCT patients, detailed flow cytometric analyses and next generation sequencing of the δ and β chain were performed. Furthermore, we performed 5' single cell RNA sequencing (scRNAseq) on patient samples ~1 year after SCT. We included patients that had undergone an αβT cell depleted allo-SCT (n=3) or T cell replete allo-SCT (n=3) and experienced a CMV reactivation after allo-SCT.
Results: In αβT cell depleted allo-SCT recipients both $V\delta2^-$ and $V\delta2^+$ γδT cells reconstituted early, reaching reference values within the first 100 days. CMV reactivation enhanced recovery of $V\delta2^-$ T cells and $CD8^+$ αβT cells. $CD8^+$ αβT cell and $V\delta1^+$ γδT cell expansion after CMV reactivation was driven by $CD27^{low}$ $T_{effector}$ cells. Interestingly, we observed that the dominance of Vδ2- γδT cells within the γδT cell repertoire lasted up to 3 years after allo-SCT, resulting in an inversed ratio of Vδ2+/Vδ2- γδT cells and associated with slow $CD4^+$ αβT cell reconstitution. This was confirmed by 5' scRNAseq analyses 1 year post allo-SCT, where the major γδT cell population was Vδ2- and $CD4^+$ αβT cells were scarce.
Conclusion: Early immune reconstitution after αβ T cell depleted allo-SCT results in a marked expansion of Vδ2- γδT cells and CD8+ αβT cells, which is further increased by CMV reactivation. Moreover, the inversed ratio of Vδ2+/Vδ2- γδT cells in our cohort suggests Vδ2- γδT cells might contribute as regulators to balance the immune system during the absence of CD4+ αβT cells.