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IMMUNE RECONSTITUTION AND INFECTIOUS COMPLICATIONS IN CORD BLOOD TRANSPLANT PATIENTS

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Umbilical cord blood grafts are increasingly used as a source of hematopoietic stem cells for patients with high-risk hematologic diseases. Despite appropriate CD34+ cell numbers after transplantation, immune recovery remains delayed and insufficient, and infection-related morbidity remains a leading cause of mortality in this patient group. The purpose of this study was to (i) evaluate the tempo of functional T cell immune reconstitution after cord blood transplant (CBT) using the IFNg ELIspot to measure the frequency of T cells reactive against common viruses including EBV, CMV, adenovirus (Adv), BK virus, influenza, and Respiratory syncytial virus (RSV), as well as Staphylococcal enterotoxin B from Staphylococcus aureus (Staph) at sequential timepoints pre- and post transplant, and (ii) to delineate the types of infections in the post-CBT period and how frequently they occur.

To assess immune reconstitution we prospectively isolated PBMCs from 52 CBT recipients before and then at monthly intervals after transplant. We assessed immune function by co-culturing the cells with Staph as well as pepmixes (15mer peptide libraries) spanning the viral antigens pp65 and IE1 (CMV), LMP1, LMP2, EBNA1, EBNA3a, EBNA3b, EBNA3c, BZLF1 (EBV), hexon and penton (Adv), VP1 and large T (BK virus), MP1 and NP1 (Influenza), and N and F (RSV). We were consistently able to detect T cell reactivity to Staph (median 395.5 spot forming cells (SFC)/ $2x10^5$, range 23-845.5) pre-transplant, but evidence of immune recovery was delayed until at least 6 months post-CBT with an average of 86 SFC/$2x10^5$, which increased to normal (pre-transplant levels) by approx. 9 months post-transplant. Recovery of virus-specific T cells, exemplified by CMV-specific immune reconstitution, was even more delayed with recovery to detectable levels (ave. 89 SFC/2 $x10^5$ cells) at the 9 month time point. In a small subset of patients viral reactivation appeared to accelerate recovery of T cells reactive against the infecting virus with a subsequent decrease in viral load suggesting that the reconstituting cells had direct anti-viral activity in vivo. We also retrospectively evaluated the incidence of viral, bacterial, and fungal infections after CBT in the same patient cohort. Approximately 30 of the patients that received a CBT died within two years of transplant with 18 of these patients succumbing to infection. The statistical analysis and conclusions are in progress and will be presented.

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REGULATORY T CELLS FACILITATE THYMIC RECOVERY AFTER HSCT BY DIRECTLY ENHANCING IMMIGRATION OF DONOR DERIVED THYMIC PROGENITORS

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Little is known regarding the mechanisms regulating thymic recovery after HSCT. Previously, we reported that dendritic cells (DCs) accelerate thymic recovery as well as enhance T cell reconstitution after HSCT. DCs in the thymus are capable of inducing regulatory T cells (Tregs) differentiation and Tregs are able to enhance immune reconstitution by preventing GVHD-induced damage of the thymic microenvironment. However the direct effect of Tregs on thymic recovery has not been described. Using a syngeneic transplant model with a GFP^+FOXP3 promoter, we show that Tregs are able to enhance T cell recovery after HSCT by directly enhancing the immigration of thymic progenitors. Lethally irradiated C57BL/6 (CD45.2) recipients received 10^3 (CD45.1) $lin^-sca\text{-}1^+c\text{-}kit^+$ (LSK) HSC progenitors or 10^3 (CD45.1) LSK cells along with ex vivo expanded ($CD45.1^+GFP^+FOXP3^+$) Tregs. On 4 and 7 days after HSCT, thymuses of the Tregs groups contained 1.5 and 2-fold higher number of thymocytes compared to control group, respectively. Furthermore, thymuses of the Tregs group contained 10-fold higher number of thymoctyes derived from donor LSK cells (CD45.1) compared to control ($p < 0.05$). Direct immigration of thymic progenitors was confirmed by detection of donor ($CD45.1^+$ GFP^-) thymic progenitors using immunohistochemistry. Furthermore, peripheral blood of the Tregs group contained 3.1-fold higher numbers of $CD3^+$ cells derived from donor LSK (CD45.1) cells compared to the control group. Lastly, we tested whether the addition of Tregs is able to facilitate BM as well as thymic engraftment. Lethally irradiated recipients received limiting doses of LSK cells alone or LSK along with Tregs. One month after HSCT, survival of the Tregs group was significantly higher than control (50% versus 0%) ($P = .04$). Similar to the thymus, we observed significant 7.2-fold increase in the number of donor engraftment in the Tregs group compared to control. The combined data suggest that Tregs plays a direct role in facilitating thymic and BM recovery to enhance immune reconstitution after HSCT.

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DONOR CD4 CELLS INDUCE HOST MICROENVIRONMENT INFLAMMATION AFTER NONMYELOABLATIVE HEMATOPOIETIC CELL TRANSPLANTATION WHICH SUPPRESSES HEMATOPOIESIS AND CONTRIBUTES TO GRAFT REJECTION

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The toxicity of allogeneic hematopoietic cell transplantation (HCT) is reduced by nonmyeloablative (NM) conditioning; however, persistent host cells can exacerbate graft rejection. To study the dynamics of hematopoietic reconstitution after NM radiation in a minor-mismatched strain combination with high levels of engraftment resistance, BALB.K mice were given purified hematopoietic stem cells (HSC: cKit+Thy1.1loSca1+Lin-) +/- T cells (TC) from AKR/J donors. Recipients of pure HSC or HSC+CD8 TC regularly achieved stable mixed chimerism. Mice given HSC+CD4+CD25- TC (CD4c) failed to engraft, displayed BM hypocellularity compared with HSC recipients (median 3.8 vs 13.3×10^6 cells/2 legs; $p = 0.0003$), and lymphopenia (< 5% vs > 40%) at 2 weeks (w) post-HCT. While the BM of mice given HSC alone had all maturation stages of stem and progenitor cells (HSC: cKit+Sca1+Lin-Flt3-CD34-CD150+ [LT = long-term]/CD150- [ST = short term]; multipotent progenitors, MPP: cKit+Sca1+Lin-Flt3+/-CD34+/-), recipients of HSC+CD4c lacked MPP, and their population of ST (> LT) HSC was enlarged 2w post-HCT. These ST HSC were arrested in G0/1, but promptly reconstituted multilineage hematopoiesis when infused into unconditioned secondary $Rag2\gamma c{-}/{-}$ recipients. At 2w post-HCT the BM, but not the spleen, of mice given HSC+CD4c contained increased proportions of CD4 TC that expressed high levels of CD69 (host > donor) and secreted IFNγ (donor: median 45%; host: 13% [$p < .001$]; < 10% in all other controls). Further, 1-2w post-HCT the BM dendritic cell (DC) pool in mice given pure HSC was dominated by tolerogenic plasmacytoid DC (CD11c+B220+), which were lacking in recipients of HSC+CD4c. Rather, the BM of the latter contained primarily myeloid DC (CD11c+Mac1+), which strongly expressed MHCII, CD40, CD80, and secreted IL-12. We evaluated several other MHC-matched strain combinations, which demonstrated lower levels of cytokine production and less impaired hematopoiesis. Overall, our data suggest that interactions between allo-activated donor CD4 TC and residual host cells create an inflammatory environment involving the IL-12/IFNγ axis which results in inhibition of hematopoiesis with evidence of HSC quiescence. In contrast to the general view that TC augment donor HSC engraftment, we observed improved donor chimerism in recipients of pure HSC and postulate that this lack of CD4 TC driven inflammation may result in superior immune reconstitution after NM conditioning.