demonstrated that extrathymic T cell development occurs in BMT recipients, especially MLN, and results in functional T cells with a broad TCR repertoire.

223

FLT-3 LIGAND ENHANCES THYMPOIESIS IN HEMATOPOIETIC STEM CELL TRANSPLANTS (HSC) INVOLVING AGED DONOR AND RECIPIENT MICE BY INCREASING SURVIVAL AND TRAFFICKING OF EARLY THYMOPOIETIC PROGENITORS

Williams, K.M., Moore, A.R., Gress, R.E. National Institutes of Health, Bethesda, MD

Impaired thymopoiesis may contribute to graft-versus-host disease and poor clearance of infections. Early thymic precursor frequency is a point of regulation for thymus reconstitution; however, it is unknown if decreased thymus recovery with age is due to the absence of thymus elements or marrow precursors.

To address this, we investigated whether marrow thymus precursor frequency declines with age, contributes to poor aged thymus recovery, and can be enhanced with FMS-like tyrosine kinase 3 (Flt-3), a growth factor for HSC. Total number of HSC (LSK, Lineage- Scf-1+ and cKit+) and Flt-3R+ LSK were equivalent in the marrow of old (>4 months) and young mice (1 month).

By ELISA, marrow concentration of Flt-3L was equivalent between old and young mice. Similarly, there was no difference in splenic LSK numbers. However, the response to exogenous Flt-3L was distinct by age. While young marrow was unaffected after Flt-3L, in aged mice, total LSK and CCR9+ thymus directed LSK increased 4 fold (p < 0.05). The proportion of LSK that expressed Flt-3R decreased in the marrow of older mice in response to Flt3-L and LSK proliferation (Ki67 and BrdU) did not differ in response to Flt3-L in old or young mice, consistent with improved survival rather than proliferation. We then hypothesized that Flt-3L treated cells may also traffic to peripheral spaces, similar to GCSF. Consistent with this theory, in the spleen of old and young mice, the Flt3-R+ LSK number increased in response to Flt3-L (p < 0.05). We then used aged Flt3-L knock-out mice (Flt3-L−/−) to test whether survival and trafficking were part of the mechanism of Flt3-L. Aged Flt3-L−/− mice had significantly lower LSK and Flt3-R+ LSK in the marrow than wild type aged counterparts, consistent with a deficient survival signal. After Flt3-L exposure, although Flt3-R proportion was unchanged in the marrow, LSK numbers increased significantly in the spleen (p < 0.05) with equivalent Ki67, suggesting LSK trafficked from marrow to spleen. Aged donor Flt3-L treated marrow with lupon-treated aged host increased donor thymocytes two-fold within 5 weeks after transplantation (p < 0.05) compared to lupon alone. Collectively, these data suggest that marrow LSK milieu contributes to thymus dysfunction in older donor/host recipient pairs and that Flt-3L may improve thymus recovery by enhancing survival and trafficking of older donor HSC.

Conclusion: CD3+ CD4+ and CD3+ CD8+ divided by their age related reference values, predicted outcome early after HSCT in children and may be used in future studies and in clinical practice, after further validation of these relationships.

224

CD3+CD4+ AND CD3+CD8+LYMPHOCYTES AS BIOMARKERS PREDICTING THE LONG TERM OUTCOME OF PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS

Bartelsink, I.H.1, Beitzer, S.V.2, Knabe, C.A.J.3,4, Danhof, M.4, de Pagter, A.J.1, Egberts, A.C.G.1,2, Buemen, J.F.1,3 University Medical Center Utrecht, Utrecht, Netherlands; 2 Utrecht University, Utrecht, Netherlands; 3 St. Antonius Hospital, Nieuwegein, Netherlands; 4 5 Leiden/Amsterdam Center for Drug Research, Division of Pharmacology, Leiden University, Leiden, Netherlands

Introduction: Haematopoietic stem cell transplantation (HSCT) may be complicated by severe infectious complications, graft-versus-host disease (GvHD) and relapse/grant failures the probability thereof depends on the lymphocyte immune reconstitution. The goal of this study was to identify biomarkers of immune reconstitution in the first period after transplantation that may predict outcome.

Methods: Children transplanted between 2005-2007 in the UMCU were prospectively included. Immunophenotyping was performed every two weeks after HSCT. Several parameters of immune reconstitution in relation with survival, acute- and chronic-GvHD were studied, using a multiplicative intensity model in R: AUC, the time and maximum CD3+/CD4+, CD4+/CD8+ T-cell and CD19+ B-cell counts, the ratio between CD4+/CD8+ and the relationship between naive and memory cells in the period 0-90,0-180 and 180-360 days. Data were stratified to donor source. Relations were tested in linear, logarithmic and parabolic functions. The differences between models were compared using the P-values and diagnostics of the model. Based on the resulting model, we defined a cut off value to describe optimal survival per biomarker.

Results: 56 recipients received bone marrow, 12 received a sibling donor, while 26 patients received cord blood derived stem cells. The median age at HSCT was 5.9 years (range 0.11 – 18.1). The max nr of CD4+ cells related to their age related reference values in the first 6 months after HSCT positively predicted survival (HR 0.43, CI95% 0.29-0.63), with a cutoff point of 0.15. In Cord blood transplants the CD4/8 ratio within the first half year > 5 was a predictor for survival (HR = 0.6 CI95% 0.40-0.91). In BM/sibling transplants, the maximum number of CD8 cells related to their age related reference values > 1 positively predicted survival within the first half year (HR = 0.68, CI95% 0.46-1.01).

Conclusion: CD3+ CD4+ and CD3+ CD8+ divided by their age related reference values, predicted outcome early after HSCT in children and may be used in future studies and in clinical practice, after further validation of these relationships.

225

IMMUNE RECONSTITUTION IN HEMATOPOIETIC CELL TRANSPLANTATION PATIENTS RECEIVING ATG AS PART OF CONDITIONING REGIME

Hoegh-Petersen, M., Dhadda, M., Lin, Y., Hagel, L., Podgursky, P.J., Ugarte-Torres, A., Storek, J. University of Calgary, Calgary, AB, Canada

Introduction: Successful immunological recovery in recipients of hematopoietic cell transplantation (HCT) is important for transplant success. Use of anti-thymocyte globulin (ATG) as part of the pre-transplant conditioning has shown to decrease graft versus host disease (GvHD). However the impact of ATG on immunological recovery has not been fully studied. Here we report immune cell recovery following influencing immune cell recovery after ATG-conditioned HCT.

Patients and Methods: Immune subsets were quantified in 176 allogeneic HCT recipients receiving ATG during conditioning, at day 28, 56, 84, 180, 365 and 730 post transplantation. Following lymphocyte subsets were quantified: B cells, CD4 T cells and CD8 T cells (incl. their naive and memory/effector [mem/eff] subsets), NK cells (incl. regulatory and cytolytic subsets), monocytes and dendritic cells (DC) (incl. plasmacytoid and myeloid subsets). Day 7 serum levels of ATG were quantified by flow cytometry. Significance of associations between immune cell subset counts and factors suspected to influence them were tested using Mann-Whitney rank sum test for categorical factors and Spearman rank correlation test for continuous variable/factors.

Results: Higher recipient age was associated with lower naive CD4 and CD8 T cell counts on days 180, 365 and 730. Graft content of specific subsets positively correlated with counts of the same subset early posttransplant in the case of B cells (both naive and memory), CD4 and CD8 T cells (both naive and mem/eff), and myeloid DCs. Significant GvHD (grade 2-4 acute or moderate-severe chronic) was associated with significantly lower B cell counts (including naive and memory) on days 56-180, regulatory NK cells on days 28-56, plasmacytoid DCs on day 28-84 and higher counts of naive CD8 T cells on days 28-84. High day 7 serum ATG levels were associated with decreased CD4 and CD8 T cell counts and increased regulatory NK cell counts early, but for most subsets not late posttransplant. Graft CD34 cell count and recipient CMV serostatus showed no positive correlation with any immune cell subset count.