be a consideration in the pre-transplant evaluation. Studies are needed to further delineate the immunologic impact of donor serostatus.

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CMV SPECIFIC T CELLS PREVENT PROGRESSION FROM LOW TO HIGH LEVEL VIREMIA IN D+R+ BUT NOT D-R+ PATIENTS

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Background: Cytomegalovirus (CMV) reactivates (becomes detectable in blood) in most seropositive hematopoietic cell transplant (HCT) recipients. In some reactivating patients, low level viremia (< 50,000 DNA copies/ml plasma, ie, less than our institutional threshold for preemtive therapy) progresses to high level viremia or CMV disease, which is potentially fatal. We hypothesized that low level viremia progresses in patients with low specific T cell counts and spontaneously resolves in patients with high specific T cell counts.

Methods: In 30 CMV seropositive HCT recipients monitored weekly for reactivation by real-time PCR, blood was drawn for specific T cell counts within 4 days from the first episode of low level viremia. Fourteen patients received grafts from seropositive donors (D+R+), and 16 patients from seronegative donors (D-R+). Mononuclear cells were stimulated overnight with CMV lysate, pp65 overlapping peptides, no stimulus (negative control) or staphylococcal enterotoxin B (positive control). T cells producing IFNγ, TNFα and/or IL2 were enumerated by flow cytometry.

Results: Among D+R+ patients, counts of CMV lysate and pp65 specific CD4 T cells producing IFNγ and TNFα (and not IL2) were higher in patients with spontaneous resolution than patients with progression ($p = 0.02$ for CMV lysate, $p = 0.004$ for pp65). Also, there was an inverse correlation between pp65 specific CD8 T cells producing IFNγ and TNFα and peak viremia ($r = -0.94$, $p = 0.005$) in D+R+ patients who progressed to high level viremia/disease. In contrast, among D-R+ patients, CMV lysate and pp65 specific T cell counts were similar in patients with spontaneous resolution and patients with progression, and there was no correlation between specific T cell counts and peak viremia.

Conclusion: CMV specific T cells play a role in preventing progression from low to high level CMV reactivation/disease in D+R+ patients. Other immune mechanisms (eg, NK cells?) play the role in D-R+ patients.

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ASSESSMENT OF IMMATURE RETICULOCYTE FRACTION AS AN EARLY PREDICTOR OF MARROW ENGRAFTMENT AFTER HEMATOPOIECTIC STEM CELL TRANSPLANTATION

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Introduction: Hematopoietic stem cell transplantation (HSCT) has been used as a treatment for hematologic malignancies and non-malignant disorders. The success of engraftment is important for determining the direction of treatment and the prognosis of patients. Various methods of early assessment have been proposed. The purpose of this study was to evaluate immature reticulocyte fraction (IRF) as an early predictor of marrow engraftment and to validate cut-off values for IRF in pediatric patients.

Patients and Methods: Retrospective analysis was performed on 172 patients including 108 males (62.8%) and 64 females (37.2%) with a median age of 8.3 years who underwent HSCT (autologous: 87 patients, allogeneic: 85 patients) in Seoul National University Children's hospital from January 1st, 2005 to December 31st, 2008. Complete blood counts with reticulocyte parameters were measured in all patients. The day of engraftment of neutrophil was defined as the first of three consecutive days of an absolute neutrophil count > 500 /μL with GCSF support. Engraftment of platelets was defined as an unsupported platelet count over 20,000/μL for seven days.

Results: Standard neutrophil engraftment occurred after a mean of 12.1±4.3 days following HSCT and platelet engraftment occurred after a mean of 19.4±11.7 days. With regards to IRF, mean rates were as follows: above 1% after 9.6±2.6 days, above 3.5% after 10.5±3.0 days, and above 5% after 11.1±3.6 days. These differences was statistically significanct for IRF and platelets when compared with standard neutrophil recovery after HSCT. Following HSCT, the mean day on which IRF increased above 3.5% preceded the standard neutrophil engraftment day ($P < 0.001$). The predicting cut-off value of IRF was 3.5% (AUC = 0.879, 95% CI, 0.759-0.999) at 8 days after HSCT. Sensitivity and specificity were 86.7% and 82.8%, respectively.

Conclusions: Considering these results of the increase in IRF preceded that of absolute neutrophil count (ANC) or platelets in most cases, IRF was useful as an early indicator of marrow engraftment especially when observed simultaneously with the other parameters.

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EXTRATHYMIC SITES DRIVE THYMUS-INDEPENDENT DEVELOPMENT OF FUNCTIONAL T CELLS AFTER BONE MARROW TRANSPLANTATION

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T cell deficiency after bone marrow transplantation (BMT) leads to significant morbidity and mortality due to infection and malignant relapse. We have previously shown that CD4-CD8- T cell precursors (preT) generated via OP9-DL1 coculture improve thymic and peripheral T cell reconstitution when administered at the time of BMT. While the thymus supports T cell development into old age, adult BMT recipients have limited thymic function due to age-related involution and additional injury caused by transplant conditioning. We therefore hypothesized that T cell reconstitution after BMT may depend to some extent on extrathymic T cell development, which has previously been described as present but abnormal.

We found that, in addition to the thymus, preT cells engrafted in extrathymic sites, including mesenteric lymph nodes (MLN), peripheral lymph nodes (PLN) and spleen, within 24 hours of transfer into euthymic BMT recipients. We detected CD4+CD8+ (double positive, DP) thymocyte-like cells of both preT and bone marrow (BM) origin in MLN, but not PLN, BM, or spleen, suggesting T cell development in MLN. Using a novel dual reporter for bioluminescence imaging we confirmed that preT trafficked to gut-associated tissues, where they underwent NFAT activation, consistent with T cell development. In competitive reconstitution studies we found that engraftment of preT in thymic and extrathymic sites required PSGL-1, while CCR9 may be dispensable.

To better study extrathymic T cell development, we transferred preT into thymectomized or athymic BMT recipients. We demonstrated that preT and BM cells develop into both TCRαβ and TCRγδ T cells in athymic BMT recipients. As in euthymic recipients, preT engraft in extrathymic gut-associated sites and MLN support DP early after transfer into athymic BMT recipients. We found more CD4+, CD8αβ+, and CD8αα+ T cells in the spleen, PLN, MLN, and Peyer's patches of athymic BMT recipients that received preT than in nontransplanted or BM only recipients. Extrathymic-derived CD4+ and CD8αβ+ T cells are naive CD44- cells with a diverse T cell receptor (TCR) repertoire. In addition, extrathymic-derived T cells proliferated to a similar extent as thymic-derived T cells and produced IFNγ and TNFα following in vitro stimulation. In conclusion, using adoptively transferred preT, we have

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

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

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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- Sca-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 lupron-treated aged host increased donor thymocytes twofold within 5 weeks after transplantation ($p < 0.05$) compared to lupron 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.

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

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Introduction: Haematopoietic stem cell transplantation (HSCT) may be complicated by severe infectious complications, graft-versus-host disease (GvHD) and relapse/graft 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+, CD3+CD8+ T-cell and CD19+B-cell numbers, 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, logaritmic and parabolar 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.

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

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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 studied. Here we studied the factors 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 naïve 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.