Follow up for infections was censored at time of relapse or death. Hazard ratios (HR) of outcome of patients with above vs below median immune subset count at the beginning of each interval were determined. The HR was adjusted for disease stage, stem cell source, immunosuppressive therapy for GVHD, and use of antimicrobial prophylaxis. Due to multiple comparisons, HR was considered significant only if $p<0.01$.

Results: No association was found between an immune cell subset count and fatal infection, non-relapse mortality, survival, or relapse. Regarding infections, the only significant association between a day 30 subset count and day 30–80 infections was between CD4-CD8- T cells and viral infections. For the subsequent time intervals, conspicuous associations are shown in Table 1.

Immune cell subset above the median and hazard ratio of definite and bacterial infections

Subset	Bacterial infection d81-180 HR(p)	Definite infection d81-180 HR(p)	Bacterial infection d181-365 HR(p)	Definite infection d181-365 HR(p)	Bacterial infection d>365 HR(p)	Definite infection d>365 HR(p)
Total B cells	0.18 (p=0.002)	NS	0.07 (p=0.0005)	NS	NS	NS
Naive B cells	0.24 (p=0.006)	NS	0.07 (p=0.0005)	NS	NS	NS
Memory B cells	NS	NS	NS	NS	NS	0.4 (0.002)
NK cells	NS	0.45 (p=0.01)	0 (p=0.003)	0.2 (p=0.001)	NS	NS
CD4 memory/ effector cells	NS	NS	NS	0.25 (p=0.002)	NS	NS

NS = not significant.

Conclusion: In contrast to previous studies, no association was found between an immune cell subset count and fatal infection, non-relapse mortality, survival, or relapse. Associations between some immune cell subset counts and infections exist and should be further studied.

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GENERATION OF VIRUS-SPECIFIC CYTOTOXIC T LYMPHOCYTES (CTLS) RESISTANT TO THE IMMUNOSUPPRESSIVE DRUG TACROLIMUS (FK506)

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Viral infections (CMV, EBV and Adenovirus) can be fatal complications post allogeneic hematopoietic stem cell and solid organ transplantation. Administration of virus-specific CTLs, targeting individual- or multiple-viral antigens is effective in these patients. However, in those whose immunosuppressive treatment needs to be maintained to prevent GvHD or organ rejection, expansion and persistence of adoptively transferred virus-specific CTLs are impaired. FK506 is frequently used as immunosuppressive drug. Its effects depend on binding to FKBP12 proteins. Since T cells generated from FKBP12 knockout mice are resistant to the inhibitory effects of FK506, we knocked down FKBP12 using a small interfering RNA (siRNA) to generate virus- specific CTLs resistant to FK506. As a model we used EBV+ tumor and EBV-CTLs. We identified one siRNA sequence (siRNA4), that knocked down >90% of FKBP12 expression in T cell lines and virus-CTLs. We then generated 2 retroviral vectors encoding for siRNA4/eGFP and irrelevant siRNA/eGFP and used them to transduce CTLs generated from 7 EBV-seropositive donors. Transduction efficiency was 46 ± 22% for siRNA4 and 55 ± 27% for irrel.-siRNA. We measured the proliferation of transduced CTLs in the presence of FK506, in short and long term cultures. We found that in the presence of FK506, proliferation of control CTLs was significantly inhibited (by 74± 2%) as compared to siRNA4+ CTLs (41 ± 4%). In long term cultures, modified CTLs were stimulated weekly with EBV+ cells, with or without FK506 (5ng/ml) and low dose IL-2 (20U/mL). The proportion of siRNA4+ CTLs increased over time not only as a percentage of GFP+ cells (from 46 ± 22% to 89 ± 5% after 5 stimulations) but also numerically (34 median fold expansion, range 5–60). In contrast, control CTLs did not show any selection in culture, as the percentage of GFP+ cells remained unchanged (from 56 ± 27% to 57 ± 23%) and CTLs ceased to proliferate (2 median fold expansion, range 0–5). Finally, we found that siRNA4+ CTLs retained their MHC-restricted cytotoxicity against EBV+ cells (66 ± 22% lysis at an E:T ratio of 20:1). Modified CTLs also maintained their production of IFN-γ in response to EBV-peptides, as assessed by ELIspot assays. In conclusion, we have developed a strategy that produces virus-specific CTLs resistant to FK506. This strategy may be beneficial to improve virus immune reconstitution in patients post transplant, despite ongoing immunosuppresion.

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ASSOCIATION BETWEEN HLA-E POLYMORPHISM AND SEVERE FUNGAL INFECTIONS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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HLA-E is a non classical HLA class I molecule that interacts with NK cell CD94/NKG2 family receptors as a major ligand playing a dual role in NK cell modulation and T cell activation, either inhibiting or triggering the citotoxicity by these cells. These roles indicate the involvement of HLA-E antigen in host-immune response against pathogens. The aim of this study was to evaluate whether the HLA-E alleles have functional impact on the incidence of bacterial, viral and fungal infections in patients who have undergone an HLA identical sibling myeloablative hematopoietic stem cell transplantation (HSCT). One hundred and seven patients and their respective donors were included in this study. HSCT was performed from 1999 to 2006, with a minimum of 6 months follow up. The distinction between HLA-E*0101 and HLA-E*0103 alleles was done either by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) and polymerase chain reaction – sequence specific primer (PCR-SSP). The predictive effect of HLA-E genotype (HLA-E* 0101/ E*0101, E* 0101/E* 0103 and E*0103/ E*0103) was assessed as variables. Univariate analysis using death as a competing risk were done to evaluate the association between HLA-E polymorphism with first infections episodes. HLA-E genotype showed no association with severe bacterial infection or viral infections. However, in first severe fungal infection episode HLA-E* 0101/ E*0101 genotype was found to have a protective effect ($P = 0.01$), since none of the patients who had severe fungal infections had this haplotype. In conclusion, it is possible that HLA-E genotype polymorphism fulfills a related function to fungal infection after HSCT, although these are preliminary data and need further confirmation.

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A ROLE FOR IRAK-M IN PGE_2-INDUCED IMMUNOSUPPRESSION POST-BONE MARROW TRANSPLANT

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Rationale: Following bone marrow (BM) transplantation (BMT), patients are susceptible to lung infections. In a mouse model of syngeneic BMT, we have shown BMT alveolar macrophages (AMs) and alveolar epithelial cells (AECs) overproduce prostaglandin E2 (PGE_2) relative to non-transplant controls. Overproduction of PGE_2 significantly impairs neutrophil killing and inhibits both phagocytosis and killing in AMs. These defects are also associated with diminished TNF-alpha production. However, a mechanism by which PGE_2 overproduction suppresses lung innate immune cell function post-BMT is unknown. As interleukin-1 receptor associated kinase (IRAK)-M is a known inhibitor of MyD88-dependent-

TLR signaling and AM function, we sought to determine whether IRAK-M was involved in PGE_2-induced immunosuppression post-BMT.

Methods: IRAK-M protein and mRNA expression were measured in AMs from control and BMT mice. Additionally, control AMs cultured in the presence or absence of 100 nM PGE_2 were analyzed for IRAK-M expression. To study the role of IRAK-M post-BMT, we transplanted either wild-type (WT) BM or IRAK-M-/- BM into WT recipients. Levels of PGE_2 were measured in AMs five weeks post-BMT. BMT and control mice were also challenged with P. aeruginosa infection and bacterial burden was measured in the lung and blood 24 hours later.

Results: BMT AMs displayed elevated IRAK-M expression relative to non-transplant control AMs. Treatment of control AMs with PGE_2 upregulated IRAK-M mRNA and protein expression. Despite overproduction of PGE_2, IRAK-M-/->WT BMT mice displayed improved host defense against P. aeruginosa lung infection compared to WT>WT BMT mice.

Conclusions: PGE_2 may impair host defense against P. aeruginosa in BMT AMs via upregulation IRAK-M. Strategies to limit IRAK-M elevation post-BMT may prove efficacious in limiting infections post-BMT.

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PLASMACYTOID CD123+ DENDRITIC CELL RECOVERY AND REDUCED ACTIVATION STATE OF CIRCULATING CD8+ T CELLS PREDICT SURVIVAL AFTER UNRELATED CORD BLOOD TRANSPLANT (UCBT)

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Background: Reconstitution of adaptive immunity is critical for long term survival following HCT. UCBT is a suitable option for those who lack HLA-matched sibling donors. Antigen presenting dendritic cells (DC) and lymphocytes are critical for acquiring protective immunity. Infections remain the major cause of TRM. In the COBLT study, successful recovery of antiviral cellular immunity was associated with a reduced rate of relapse.

Methods: Between July 2005, and December 2007, 95 children with full donor chimerism following myeloablative conditioning and a single cord UCBT were assessed for reconstitution of DC and T cell subsets, B and NK cells at 3, 6, 12, 24, and 36 months after UCBT to analyze their impact on overall survival (OS).

Results: 52 and 43 children were transplanted for non-malignant and malignant diseases, respectively. The median age was 2.7 years, 55 patients were male, 38 (40%) were 4/6, 39 (41%) were 5/6 and 18 (19%) were 6/6 HLA match. The median infused TNC and CD34+ cell dose was 7.2×10^7/kg and 1.9×10^5/kg, respectively. Of the 95 patients, 6 died before day 180, 11 died between day 180 and 365 [1-year survival probability 81.7% (95% CI 72.2%-88.2%)], and 8 died between day 365 and 730 [2-year survival probability 69.1% (95% CI 57.1%-78.3%)]. In an univariate analysis of OS post-transplant, non-malignant diagnosis (HR = 0.25, p = 0.001), HLA match of 5/6 or 6/6 (HR = 0.41, p = 0.02), absolute number of CD123+ "plasmacytoid" dendritic cells (pDC) >8 cells/ul (HR = 42, p = 0.04), %CD45RA+/CD62L+ "recent thymic emigrants" >13% (HR = 0.41, p = 0.02), %HLA-DR+/CD8+ T cells <19% (HR = 0.25, p = 0.01) were each associated with a decreased risk of death, while "myeloid" DC and other lymphocyte recovery had no statistical impact. In this cohort where all studied patients have successfully engrafted, gender, race, CMV serology, TNC, CD34+ cell dose, and TBI had no impact. In multivariate models non-malignant disease remained a predictor for better OS. (HR = 0.27, p = 0.002). Two immune parameters remained significant predictors of survival when patient and graft specific variables were included in multivariate analysis; abs# of CD123+ pDC >8 cells / ul (p = 0.05) and %HLA-DR+/CD8+ T cells <19% (p = 0.01).

Conclusion: This is the first analysis to suggest that the absolute number of CD123+ pDC >8 Cells /ul is an independent predictor of survival after UCBT. Independently, lower activation state of CD8+ T cells also correlates with superior survival.

Table 1. Univariate and multivariate analysis of overall survival (OS).

	Hazard Ratio	P-value
Univariate Model		
Gender		
Female	1.52	0.3
Male	1.00	
Disease		
Non-Malignant	0.25	0.001
Malignant	1.00	
Cell Doses		
TNC / kg	1.03	0.4
CD34+ / kg	1.01	0.9
HLA Match		
5 / 6 or 6 / 6	0.41	0.02
4 / 6	1.00	
Cell Type		
Absolute Number of CD123+ DC (pDC / ul)	0.42	0.04
CD45RA+/CD62L+ "Recent Thymic Emigrants" >13%	0.41	0.02
%HLA-DR+/CD8+ T cells <19%	0.25	0.01
Multivariate Model		
Disease		
Non-Malignant	0.27	0.002
Malignant	1.00	
HLA Match		
5 / 6 or 6 / 6	0.53	0.1
4 / 6	1.00	
Cell Type		
Absolute Number of CD123+ (pDC / ul)	0.44	0.05
HLA-DR+/CD8+ T Cells <19%	0.29	0.01

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HOMEOSTATIC MECHANISMS AFFECT RECONSTITUTION OF CD4+ FOXP3+ REGULATORY T CELLS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

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CD4+FoxP3+ regulatory T cells (Treg) play a critical role in immune tolerance after allogeneic HSCT but the homeostatic mechanisms that affect Treg reconstitution post-HSCT have not been identified. To address this issue we simultaneously examined phenotypic and functional characteristics of Treg and CD4+FoxP3- conventional T cells (Tcon) in peripheral blood after allogeneic HSCT (n = 16, median 10 months post-HSCT). Post-HSCT Treg expressed higher levels of mitotic marker Ki-67 than Tcon (median 5.2% vs 1.5%; p<0.0001) and Treg from normal donors (median 5.2% vs 2.5%; p = 0.03). %Ki-67+ Treg was inversely correlated with peripheral CD4 count (r = -0.86, p<0.0001). To assess survival of Treg, we purified 4 different CD4+ T cell subsets by cell sorting (CD45RA+ Tcon, CD45RA- Tcon, CD45RA+ Treg and CD45RA- Treg) from 4 healthy donors, 8 patients post-autologous and 8 post-allogeneic HSCT. Each subset was cultured with or without agonistic FAS antibody and apoptosis was measured using Annexin-V staining. Anti-FAS rapidly induced apoptosis of CD45RA- Treg from patients (median 25.3%) while all other Treg and Tcon (median 4.5%, p<0.0001) subsets were relatively resistant to apoptosis. The degree of memory CD45RA- Treg apoptosis is equivalent after autologous and allogeneic HSCT, but inversely correlated with the peripheral lymphocyte count in the patient (r = -0.54, p = 0.03). The effect of lymphopenia was also evaluated by prospectively monitoring Treg and Tcon recovery 3–24 months post-HSCT in 46 patients who received peripheral stem cell grafts from HLA-identical donors after myeloablative conditioning. In 27 patients with >220/ul CD4 T cells at 6 months, both Tcon and Treg gradually increased over the subsequent 18 months. In contrast, in 19 patients with <220/ul CD4 T cells at 6 months, Tcon gradually increased but Treg decreased over the subsequent 12–24 months. These results indicate that Treg reconstitution is characterized by increased peripheral proliferation but this is counterbalanced by increased sensitivity to apoptosis. These characteristics are more