View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

cells peak around day +30. Median number of NK cells on day +30 was 420/ml (range; 161–1167) in CD3/CD19 depleted group compared to 234/ml (range; 9- 644) in the CD34+ selected group. On day +100 the median number of NK cells/ml were 210 (range; 25–350) versus 68 (range; 50–123) (p = 0.02) respectively. Recipient NK cell phenotype and function were analyzed and compared to donor NK cells. We observed an increase of CD56^{bright} NK cell subset in both groups. The median percentage of CD56^{bright} cells among NK cells in peripheral blood was 21.4% (range; 7.8–46%) for patients receiving a CD3/CD19 depleted graft and 33% (range; 17.7–62%) ($p<0.05$). On day +100 the median percentage of CD56^{bright} cells were 13.4% (range; 4.6–22%) and 37.5% (range; 22.5–61%) (p = 0.001) respectively. Patients NK cell cytotoxicity was lower at time of engraftment (3%) compared to their respective donors (20%) ($p < 0.05$) and reached the donor values by day +100. We observed a diminished KIR expression on KIR2DL1, KIR2DL2 and especially in KIR3DL1. We also observed a lower expression of activating receptors NKp30 and NKp44 and in the activating C-type lectin receptor NKG2D. The expression of CD69 was also lower compared with their respective donors. These results suggest that NK repertoire early after unrelated transplantation is determined by the imbalance between CD56^{bright} and NK CD56^{dim} subsets. This imbalance is higher in patients undergoing unrelated transplantation using CD34+ selected graft compared to those patients receiving a CD3/CD19 depleted graft.

390

IMMUNE RECONSTITUTION OF REGULATORY T-CELLS FOLLOWING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

Perez-Garcia, A.[1], *Cabezudo, E.*[2], *Lopez-Jimenez, J.*[3], *Marugan, I.*[4], *Peralta, T.*[1], *Arnan, M.*[1], *Ramos-Oliva, P.*[3], *Benet, I.*[4], *Lopez, S.*[1], *Mestre, M.*[2], *Odriozola, J.*[3], *Solano, C.*[4], *Duarte, R.F.*[1] [1] *Catalan Institute of Oncology - Duran i Reynals, Barcelona, Spain;* [2] *Hospital Universitari de Bellvitge, Barcelona, Spain;* [3] *Hospital Ramón y Cajal, Madrid, Spain;* [4] *Hospital Clínico Universitario, Valencia, Spain*

The regulatory function of CD4^{+}CD25^{high}CD127^{dim} T-cells (Tregs) has been associated with the risk of disease relapse, with GVHD in the allogeneic HSCT setting, and with the immunomodulatory capacity of autologous HSCT for autoimmune disorders. Despite their potentially important role in HSCT, their patterns of immune reconstitution postransplant remain unknown. In this study, we have analyzed the kynetics of immune reconstitution of Tregs in 42 adult (median age 56, 28–74), 27 male and 15 female, recipients of an autologous peripheral blood HSCT following conditioning with Melphalan-200 (multiple myeloma, n = 31) or BEAM (NHL, n = 11) from May 2006 to October 2007. Peripheral blood samples were prospectively collected prior to and at various time points after HSCT. Tregs were defined by their immunophenotype (CD3^{+}CD4^{+}CD25^{high}CD127^{dim}) and are expressed as a percentage of total CD4+ T-cells. FoxP3 and CTLA4 mRNA expression was performed in duplicate with 500ng mRNA in a LightCycler with TaqMan probes, and is presented in arbitrary units as a normalized ratio to the level of expression of β2-microglobulin. Immune reconstitution of Tregs occurs unexpectedly early after HSCT. The percentage of Tregs triples the pretransplant baseline level at only two weeks postransplant (6.35% vs 2.17%; $p<0.001$; table 1). Tregs remain high at day +90 when lymphocyte count recovery starts taking place. The level of early Tregs expansion at day +14 showed a strong correlation with the baseline Tregs level prior to HSCT (r = 0.74; p = 0.029). Markers of Tregs regulatory function such as FoxP3 (10.32 vs 3.0; p = 0.003) or CTLA4 (13.21 vs 6.35; p = 0.082) outline a profile of mRNA expression that mirrors the early expansion of Tregs, providing additional evidence of a true functional immune reconstitution of Tregs early after SCT.

Table 1

Time Points	CD3+ Lymph (×10e9/L)	Tregs (% of CD4+)	FoxP3 (β2-ratio)	CTLA4 (β2-ratio)
Pre-HSCT	1.46 ± 0.67	2.17 ± 0.96	3.00 ± 4.78	6.35 ± 6.64
Day +14	1.04 ± 1.28	6.35 ± 1.45	10.32 ± 9.56	13.21 ± 13.65
Day +90	2.07 ± 1.15	5.87 ± 2.13	2.42 ± 1.85	6.22 ± 4.49
ANOVA	0.061	0.001	0.003	0.082

values in table are mean±S.D.

This is the first study to analyze the kynetics of immune recovery of Tregs after autologous HSCT, and it shows that it takes place as early as two weeks into the procedure. Although FoxP3 and CD25-expression can be induced in non-regulatory T-cells by early allorreactive phenomena after allogeneic SCT, this should not be a confounding factor or compromise the analysis in the autologous setting. Understanding the early kynetics of Tregs postransplant reconstitution offers potential targets for immunomodulatory therapeutic strategies at the early phase of Tregs expansion postransplant or even at their baseline level which correlates with their subsequent expansion.

391

DIFFERENTIAL GENOMIC EXPRESSION OF NK RECEPTOR (NKR) IN CORD BLOOD (CB) CD56^{DIM} VERSUS PERIPHERAL BLOOD (PB) CD56^{DIM} NK CELLS: IMPLICATION FOR IMMATURITY IN CORD BLOOD NK CD56^{DIM} INNATE IMMUNITY

Shereck, E.[1], *Day, N.S.*[1], *Ayello, J.*[1], *McGuinn, C.*[1], *Satwani, P.*[1], *Lewis, D.*[1], *van de Ven, C.*[1], *Wapner, R.J.*[2], *Lim, M.*[3], *Cairo, M.S.*[1,4,5] [1] *Morgan Stanley Children's Hospital New York Presbyterian, Columbia University, New York, NY;* [2] *New York Presbyterian Hospital, Columbia University, New York, NY;* [3] *University of Michigan, Ann Arbor, MI;* [4] *Morgan Stanley Children's Hospital New York Presbyterian, Columbia University, New York, NY;* [5] *Morgan Stanley Children's Hospital New York Presbyterian, Columbia University, New York, NY*

NK cells are characterized by absent CD3 but expression of CD56^{dim} (90%, cytotoxic) and CD56^{bright} (10%, mediator). NK cells may contribute to the immaturity in cord blood innate and adaptive immunity, and play an important role in the GVL effect post CBT. We previously demonstrated the ability to *ex-vivo* expand CB into NK subsets with profound NK *in-vitro* and *in-vivo* cytotoxic activity (Ayello/Cairo BBMT 2006). We further observed differential protein profiling in CB vs PB CD56^{dim}, in which NKG2A was overexpressed (Shereck/Cairo, ASH 2007; ASPHO 2007; AACR 2007). The differential proteins also included IP3R type 3, NCR3, MAPKAPK5, Notch 2, PLEK, and NF-X1. In this report, we compared the genomic expression pattern in CB vs PB CD56^{dim}. CB and PB NK cells were isolated indirectly by magnetic separation from non-NK cells. Then, the CD56^{+} CD16^{+} NK cells (CD56^{dim}) were selected with CD16 (FCGR3) MicroBeads. For genomic studies, total RNA was isolated and reverse transcribed to cDNA using T7-Oligo (dT) primer. cRNA was Biotin-labeled by *in vitro* transcription. Fragmented biotin-labeled cRNA was hybridized to GeneChip U133A_2 (Affymetrix). Data were analyzed using Agilent GeneSpring. Signal intensities were compared using one way ANOVA and Welch Test for statistical analysis. There were 193 and 222 genes over and under expressed at the genomic level between CB vs PB CD56^{dim} NK cells, respectively. CB vs PB CD56^{dim} significantly overexpressed NKG2A (2.14F), CD16b (2.46F), KIR2D (2.13F), NKp44 (NCR2; 2.62F), PBX1 (4.29F), ENPEP (3.93F). There was no significant difference in NKR gene expression of CD16a, CD161, NKG2C, and NKp46 in CB vs PB CD56^{dim}. CB vs PB CD56^{dim} underexpressed IP3R (1.32F), MAPKAPK5 (1.77F), NCR3 (1.24F), ACACB (3.23F), BBS1 (2.00F). These results suggest that NKR protein product levels in CB CD56^{dim} may be directly regulated at the translation level. The differential gene expression of IP3R, ENPEP, PBX1, and MAPKAPK5 between CB vs PB suggests the involvements of IP_3 and calcium ions in NKR signaling pathways. The potential regulators of this process may include PBX1, ENPEP, ACACB, and BBS1. We conclude that genomic differences between CB vs PB CD56^{dim} may play an important role in regulating NKR signaling pathway, and thus contribute to disparate cytotoxic activity between CB vs PB and suggest a possible explanation for immaturity of cord blood innate and adaptive immunity. (The first two authors contributed equally.)

392

HUMAN HERPES VIRUS TYPE 6 REACTIVATION AFTER HAEMATOPOIETIC STEM CELL TRANSPLANTATION: SUPERIOR SPECIFIC HHV6 T-CELL RESPONSES ARE ASSOCIATED WITH POOR CLINICAL OUTCOME

de Pagter, A.P.J.[1], *Boelens, J.J.*[1], *Tesselaar, K.*[3], *Nanlohy, N.*[3], *Bloem, A.*[3], *Schuurman, R.*[2], *van Baarle, D.*[3] [1] *University Medical*