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DIFFERENT GVHD CLINICAL PROFILE AMONG PATIENTS WHO RECEIVED CORD BLOOD, COMPARED TO THOSE WHO RECEIVED MARROW OR PERIPHERAL BLOOD

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It is well known that transplants from cord blood stem cells have lower incidence of graft-versus-host disease, which allows procedures with more HLA disparities when compared to marrow. The objective of this study, however is to determine if there is a different clinical expression of GVHD beteween receptors of peripheral blood/marrow versus cord blood.

Patients and Methods: From October 1979 to June 2007, 1530 patients were transplanted at the BMT center of the Federal University of Parana. We retrieved the database of our institution and divided the patients into two categories. Group one: 1390 patients who received bm or pb as stem cell source. Group 2: 140 patients who received cord blood. Main differences between the two goups are listed at the table below. Statystical Analysis: Kaplan Meier was used for survival and Fisher test for comparison of categoric variables. **Results:** See table 1. **Conclusions:** 1. There was a lower incidence of lung and oral involvement by chronic GVHD and a higher incidence of gut involvement on patients who received CB as compared to PB and Marrow. Furthermore, we did not see any case of bronchiolitis obliterans in the CB group. 2. There was less oral and more gut and liver involvement by acute GVHD in the CB group. 3. Overall incidence of chronic GVHD was lower for the cord blood group. There were a higher frequency of acute GVHD grade II-IV among CB recipients, possibly because of the increased frequency of HLA disparity.

CB × Marrow/PB: acute and chronic GVHD

Characteristics	Group 1 (CB)	Group2 (BM/PB)	P value
N	140	1390	
Age (M) years	6	20	
SCT donor			0.0001
related	18	1235	
unrelated	122	155	
HLA			0.0001
compatible	34	1296	
1 mismatch (A, B, DR)	47	89	
other	59	5	
Male/Female	83/57	841/549	
Sex mismatch (male recipient/female donor)	40 (29%)	289 (21%)	
A-GVHD	51 (36%)	350 (45%)	0.0385
Grade III-IV	26/51 (51%)	113/350 (32%)	
Skin	41 (80%)	297 (85%)	
Liver	12 (24%)	141 (40%)	0.0212
Oral	2 (4%)	31 (8%)	
Lung	0	4 (1%)	
Gut	20 (15%)	89 (25%)	0.0440
C-GVHD extensive	13/95 av (14%)	238/1115 av (21%)	
severe	4/13 (30%)	95/238 (40%)	
C-GVHD organ			
Skin	7 (53%)	106 (45%)	
Liver	10 (77%)	92 (39%)	0.0084
Oral	2 (15%)	108 (45%)	0.0434
Lung	1 *(7%)	36 (15%)	
Gut	6 (46%)	48 (20%)	0.0376
Survival (%)	68 (49%)	721 (52%)	
Survival (M) days	196 (0–5231)	1028 (0–9078)	

*BOOP.

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ALLOSPECIFIC EFFECTOR MEMORY T CELLS HAVE DECREASED ABILITY TO INDUCE ACUTE GVHD

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Several different groups have independently demonstrated that non-allospecific memory T cells do not induce GVHD. The data related to the ability of allospecific memory T cells to induce GVHD have not been conclusive. In order to study this question more definitely, we have developed a novel GVHD model using TEa TCR transgenic mice as donors. TEa $CD4^+$ TCR transgenic mice (C57BL/6 background) express a TCR that recognizes the peptide ASFEAQGLANIAVDKA in the context of $I\text{-}A^b$. This peptide corresponds to positions 52–68 from the alpha-chain of I-E class II molecules and is expressed in all APCs from $H\text{-}2^b/I\text{-}E^+$ strains such as CB6F1 mice. Titration experiment demonstrated that as few as 1×10^5 TEa cells were able to induce lethal GVHD in lethally irradiated CB6F1 recipients, making it an ideal model to study the ability of allospecific memory T cells to induce GVHD. Because sufficient numbers of allospecific memory phenotype T cells could not be obtained from the TEa mice or C57BL/6 CD45.1 mice containing TEa cells after priming, we chose the $Rag1^{-/-}$ model. TEa cells were first parked in $Rag1^{-/-}$ mice and then were immunized with irradiated CB6F1 cells. Eight weeks later, TEa cells were harvested from the spleens and effector memory T cells were obtained after depletion of $CD62L^+$ cells. Many TEa cells that were parked in the $Rag1^{-/-}$ mice but were not immunized with alloantigens also obtained the effector memory T cell phenotype. These cells were termed as "unprimed TEM" while those from primed animals were termed as "primed TEM". Both primed and unprimed effector memory TEa cells were able to respond to alloantigens in vitro. However, neither primed nor unprimed effector memory TEa cells was able to induce lethal GVHD in vivo and all animals in the effector memory T cell groups survived more than 100 days post transplantation. In contrast, all naive T cell recipients developed lethal GVHD and died within 35 days after transplantation. These data demonstrate that, similar to non-allospecific memory T cells, allospecific effector memory T cells also have decreased ability to induce GVHD when compared with naive T cells. This is an excellent model for us to study the unique immune response mediated by allospecific memory T cells in GVHD.

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SURVIVAL AFTER EXTRACORPOREAL PHOTOPHERESIS (ECP) FOR TREATMENT OF GRAFT-VERSUS-HOST DISEASE (GVHD) IS PREDICTED BY GVHD CLASSIFICATION AS PROPOSED BY NATIONAL INSTITUTE OF HEALTH (NIH) CONSENSUS CRITERIA

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ECP is used in GVHD treatment with variable response. The clinical phenotype of GVHD associated with ECP responsiveness is not clear. Subtypes of GVHD proposed by NIH consensus criteria affect survival after allogeneic stem cell transplant (SCT) (BBMT 2007 Oct; 13(10):1207–15). We hypothesized that survival after ECP will be determined by NIH subtypes of GVHD.

Methods: Review of patients undergoing ECP for GVHD treatment was done. 55 patients (pts.) were identified and GVHD was reclassified using NIH criteria. Pts. with acute GVHD (aGVHD) were graded using Glucksberg criteria. Pts. with overlap or classic chronic GVHD (cGVHD) were scored using NIH criteria. Overall survival (OS) was measured from starting ECP. ECP indication was steroid dependency or refractoriness. **Results:** Classic aGVHD (26%), recurrent aGVHD (7%), overlap cGVHD (16%) and classic cGVHD (51%) accounted for the subtypes. Pts. started ECP at a median of 216 days after SCT (range, 41 to 2946). The median number of GVHD recurrences prior to ECP start was 2 (range, 0–6). Pts. with classic cGVHD started ECP significantly later (145 days vs. 53 days, $P < 0.001$), continued it for a longer time

(245 vs. 104, P = 0.004) and received more treatments (13 vs. 8, P = 0.006), compared with non-classic cGVHD. The steroid dose (mg/kg) prior to ECP was lower in classic cGVHD (0.43 vs. 1.21, P < 0.001). 25 of 38 pts. (66%) with classic cGVHD were on ≤1 mg/kg of prednisone at ECP start, and 14/17 (82%) of pts. with non-classic cGVHD were on > 1 mg/kg of steroids (P = 0.001). For the entire cohort, the steroid dose at month 2 of ECP was significantly less (0.81 vs. 0.38, P = 0.004). In pts. with classic cGVHD, there was a trend in decrease in skin subscale scores after 2 months of ECP. In univariate analysis, OS was superior for classic cGVHD compared with other subtypes (median survival not reached vs. 78 days, P < 0.001; 1-yr OS 65% vs. 10%). OS was better for pts. with steroid dose ≤ 1 mg/kg at start of ECP compared with pts. on higher dose steroid (median survival not reached vs. 69 days, P < 0.001, 1-yr OS 65% vs. 0%). Using Cox regression (adjusted for steroid dose) non-classic cGVHD was an independent prognostic feature for poor survival (HR 4.72, 95% CI 1.84–12.41, P = 0.001). **Conclusion:** Pts. with classic cGVHD had a superior survival after ECP compared to other NIH subtypes. Survival after ECP with other GVHD subtypes is poor and combination of novel steroid sparing agents with ECP need to be explored.

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THYMOGLOBULIN BINDS NATURAL KILLER CELLS AND INDUCES ACTIVATION AND INTERFERON-γ PRODUCTION

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Background: Antithymocyte globulin (Thymoglobulin, rabbit IgG) is widely used in hematopoietic stem cell transplantation (HSCT) to prevent rejection and graft-versus-host disease (GVHD). A beneficial effect of natural killer (NK)-cell alloreactivity on HSCT outcome has been described, but only in patients receiving ATG. We therefore investigated the *in vitro* effects of ATG on purified NK cells. **Methods:** ATG binding to human NK cells and their activation status were assessed by flow cytometry. NK surface targets for ATG were determined by competition inhibition assays using monoclonal antibodies. Chromium 51 (^{51}Cr) release assay, annexin V combined to 7AAD staining and CFSE staining were used to study cytotoxic activity, apoptosis/cell death and proliferation of NK cells, respectively. Interferon (IFN)-γ production was determined by ELISA. **Results:** ATG, ATG-derived Fab'2 fragments as well as rabbit IgG bound NK cells, and competed strongly with anti-CD16. ATG enhanced the expression of activation (CD69, NKG2D) and degranulation (CD107a) markers on NK cells. ATG competed with CD18 binding and decreased NK cytotoxicity, which was restored by IL-15. No effects on apoptosis/cell death and proliferation were observed. Interestingly, ATG, ATG-derived Fab'2 fragments as well as rabbit IgG strongly induced IFN-γ production. **Conclusions:** ATG binds NK cells via CD16 by its variable and constant regions. The decrease in NK cytotoxic activity *in vitro*, which may be explained by CD18 binding, is restored by IL-15, and contrasts sharply with the induction of activation, degranulation and IFN-γ production. These data support the hypothesis that ATG treatment is required to observe the beneficial effect of NK cell alloreactivity in HSCT.

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EXTRACORPOREAL PHOTOCHEMOTHERAPY (ECP) FOR STEROID-REFRACTORY GRAFT-VERSUS-HOST DISEASE (GVHD) IN LOW-WEIGHT PEDIATRIC PATIENTS. CHANGES IN L-SELECTIN EXPRESSION BY T LYMPHOCYTES AND CLINICAL OUTCOME

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Extracorporeal photochemotherapy (ECP) is an emerging treatment modality for steroid-refractory GVHD. The mechanisms by which ECP works are still not fully understood, and modulation of dendritic cell subpopulations, a shift of cytokine profile from Th1 to Th2 and an increase of T-cell regulatory (Treg) cells have been related to the ECP beneficial effect. We analyzed the clinical outcome and the effect of ECP on the T lymphocyte subsets of 11 children with steroid-refractory GVHD. ECP was performed by a continuous-flow cell separator (COBE Spectra). We studied the L-selectin (CD62L) and the CD45RA expression on CD4 and CD8 lymphocytes by flow cytometry. We compared the proportion of each of these 4 subpopulations, as well as the L-selectin positive and L-selectin negative ones, in samples collected from peripheral blood before the first (PRE) and after the last (POST) ECP procedures. Results are shown in Table 1.

Complete response (CR) was achieved in six cases. Skin involvement responded in all cases. ECP was associated with minimal side effects. There was a significant increasing in the CD4/CD8 ratio. Central memory (CM) cells decreased and effector memory (EM) cells increased with ECP in CD4 and CD8 T–cell subsets. The proportion of L-selectin expressing T lymphocytes significantly diminished after ECP, both in CD4 and in CD8 cells. L-selectin is an important T-cell homing receptor for T-cell entry into lymph nodes via high endothelial venules. Expression of CD62L is rapidly lost following T-cell receptor activation, leading to exit from the lymph node into the periphery and sites of inflammation. $CD62L^{neg}$ and $CD62L^{pos}$ also differ in their functional abilities, such as cytokine secretion and cytolytic potential.

Our results suggest that ECP may have an impact in the trafficking patterns of T lymphocytes, redirectioning T cells from lymphoid to extralymphoid organs.

CD4 and CD8 subsets pre and postECP

	PRE	POST	p value
CD4 subsets			
TN (CD62L+CD45RA+)	6.58 ± 2.39	6.18 ± 2.96	0.2
TCM (CD62L+CD45RA-)	58.17 ± 4.49	43.85 ± 4.78	0.02
TEM (CD62L-CD45RA-)	34.64 ± 4.51	46.86 ± 4.89	0.03
TD (CD62L-CD45RA+)	0.6 ± 0.18	3.11 ± 1.46	0.17
CD62Lpos	64.76 ± 4.4	50.03 ± 5.45	0.02
CD62Lneg	35.23 ± 4.4	49.97 ± 5.45	0.02
CD8 subsets			
TN (CD62L+CD45RA+)	16.95 ± 3.94	12.53 ± 4.05	0.23
TCM (CD62L+CD45RA-)	28.8 ± 4.95	12.27 ± 2.86	0.001
TEM (CD62L-CD45RA-)	46.62 ± 5.79	51.4 ± 4.22	0.14
TD (CD62L-CD45RA+)	11.17 ± 3.34	23.52 ± 4.79	0.01
CD62Lpos	45.75 ± 6.1	24.8 ± 5.34	0.01
CD62Lneg	53.8 ± 6.07	74.92 ± 5.31	0.01

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REDUCED RELAPSE RELATED DEATH (RRD) IN ALTERNATIVE DONOR TRANSPLANTS WITH EBV/CMV REACTIVATION

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Viral infections are a serious diagnostic and therapeutic problem in patients undergoing alternative donor transplants. We have analyzed 179 transplants with hematologic malignancies, ghrafted from unrelated (n = 139) or family mismatched donors (n = 40): Patients in this study were alive on day +30 and had been monitored weekly for cytomegalovirus (CMV) reactivation by CMV-antigenemia, and for epstein barr virus (EBV) reactivation by real time PCR. All patients received anti-thymocyte globulin 7.5–11 mg/kg for GvHD prophylaxis together with cyclosporin and methotrextae. The conditioniong regimen was a conventional CY-TBI (n = 102) or a reduced intensity (RIC) thiotepa based regimen (n = 77). The diagnosis was acute leukemia (n = 126) or chronic lymphoid or myeloid disorder (n = 53).

Median age was 36 years (11–64) and transplants were performed between 2000 and 2006. Reactivation of CMV was seen in 78 patients (44%); EBV reactivation in 118 patients (66%). The average time to CMV reactivation was day 49 (95%CI day 29–69) and for EBV it was day +78 (95%CI day 50–105). With an average follow up of 823 days (95%CI 717–929) the overall actuarial 5 year survival