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FACTORS PREDICTING ALLOGENEIC PERIPHERAL BLOOD STEM CELL (PBSC) MOBILIZATION AFTER G-CSF TREATMENT IN HEALTHY DONORS

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Some healthy donors may show poor mobilization response to G-CSF and poor CD34+ apheresis yields. Therefore, identifying donors at risk for poor mobilization could be of value in optimising transplantation approaches. This single centre report analyzed factors associated with PBSC mobilization and yield in a homogeneous caucasian population (n = 95; 53% males) of healthy adult donors. All donors received G-CSF dosed at 10 µg/kg/d for 5 days followed by large volume leukapheresis. 69 donors (73%) were healthy sibling donors, while 26 (27%) were healthy volunteer donors for HLA-identical unrelated transplants. All donors were undergoing their first PBSC mobilization. Donors' demographic characteristics were as follow (median; range): age, 47 (18–81) y., weight, 69 (43–106) kg, height, 170(150–187) cm, body-mass index (BMI), 23.9 (15.2–35.7) kg/m^2. As per institutional policy, the targeted total number of CD34+ stem cells was between 4 and 8 ×10e6/kg recipient body weight to be collected in a maximum of 3 apheresis sessions. Overall, the median number of collected CD34+ cells was 6.25 × 10e6/kg (range, 1.7–16.6), with 16 donors (17%) yielding less than 4 × 10e6/kg CD34+ cells. In univariate analysis, female gender, lower weight, height, pre-G-CSF and post G-CSF Hb levels, and low CD34+ cell counts prior to first apheresis, were associated with significantly lower total CD34+ stem cells yields (<6.25 × 10e6/kg). In multivariate analysis, male donor gender and higher post-G-CSF CD34+ cell counts prior to the first apheresis were most strongly associated with a higher total number of collected CD34+ stem cells (OR = 6.17, 95%CI (2.39–15.93), P = 0.0001; and OR = 3.95, 95%CI (1.53–10.19), P = 0.004 respectively). Also, when considering the group of 16 "poor" mobilising donors (CD34+ stem cells yield <4 × 10e6/kg), in multivariate analysis, we found that a higher post-G-CSF CD34+ cell count prior to the first apheresis was the strongest parameter significantly associated with a higher total number of collected CD34+ stem cells (OR = 6.36, 95%CI (1.68–24.15), P = 0.006). These results indicate that a quick assessment of risk for poor mobilization response in healthy donors can be achieved through simple demographic and routine parameters. Knowledge of predictive factors for mobilization to G-CSF may be of high interest, with the development of newer mobilizing agents like CXCR4 antagonists.

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CORD BLOOD STORAGE FOLLOWING COLLECTION: IMPACT ON VOLUME REDUCTION AND CRYOPRESERVATION

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Delays from cord blood (CB) collection to processing and cryopreservation vary among CB banks and optimal CB storage length and conditions are still poorly defined. Only about 25% of the collected CB units contain a total nucleated cell (TNC) content ≥1 × 10^9, providing enough cells to transplant a 40 kg recipient. Our preliminary results suggest that CB is best stored at 4°C for up to 3 days without significant losses in cell numbers and viability. We tested whether pre-cryopreservation CB storage conditions impact cell recovery and viability after processing and cryopreservation. CB units were collected and processed and cryopreserved either on the day of collection (day 0) or after a 3-day storage at 4°C or room temperature (RT). They were volume-reduced by buffy coat separation (Optipress II) or red cell sedimentation (Hespan). Processed CB units were cryopreserved and stored for 1 month before thawing and washing, and were assessed for TNC, mononucleated cell (MNC) and CD34+ cell recovery, and CD45+ and CD34+ cell viability. Storage at RT for 3 days induced a significant loss in TNC and MNC after processing whereas storage at 4°C did not induce a significant change compared to units processed on day 0. Both Optipress II- and Hespan-processed CB units showed low CD45+ cell viabilities when stored at RT (e.g. 64 ± 10% at RT and 82 ± 8% at 4°C *vs* 98 ± 2% on day 0, p<0.002 *vs* day 0 for the Optipress group). Interestingly, CB units stored at RT for 3 days showed reduced TNC, MNC and CD34+ cell recoveries and reduced CD34+ cell viability upon thawing and washing compared to units cryopreserved on day 0 or stored at 4°C for 3 days (e.g. CD34+ cell viability in the Optipress group: 70 ± 14% at RT, 87 ± 10% at 4°C *vs* 84 ± 5% on day 0, p<0,03 *vs* day 0). These results indicate that prolonged storage at RT prior to cryopreservation can negatively impact CB cell recovery and viability after processing and upon thawing and washing of cryopreserved units.

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TREATMENT OF THREATENING REJECTION AFTER UMBILICAL CORD BLOOD TRANSPLANTATION WITH EX VIVO EXPANDED CB DERIVED T CELLS

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For patients lacking a human leukocyte antigen (HLA)-matched donor, umbilical cord blood (UCB) is a promising source of hematopoetic stem cells. Greater HLA disparity can be tolerated between the recipient and donor UCB compared with bone marrow or peripheral blood stem cells because of the naive and immature phenotype of UCB derived T cells. The risk of rejection is increased after UCB transplantation. After HLA-identical sibling or matched unrelated SCT the graft-versus-leukemia (GVL) effect may be increased by donor lymphocyte infusion (DLI) after SCT. However, after UCB transplantation DLI is not possible. This raised the question of whether ex vivo expanded CB lymphocytes (CBL) also can be used as a tool for adoptive immunotherapy after CB SCT. We have managed to establish a protocol for massive expansion of CB derived T cells making them usable in the clinic suitable for DLI after CB transplantation. We have further been able to show that the expansion protocol doesn't skew the T cell population regarding the phenotype, CD4:CD8 ratio as well as TCR usage profile measured by spectratyping. By activating the cells we have further investigated and confirmed the expanded T cells capacity to efficiently produce pro-inflammatory cytokines and respond in an allogeneic setting. We have now tried expanded CBLs in an adult patient with AML with threatening rejection after double UCB transplantation. The patient received Bu/Cy and ATG as conditioning therapy. He was transplanted with double UCB with a total nucleated cell dose of 5 × 107/kg. Both UCB units were 5/6 matched for HLA-A,-B and -DRB1 with low resolution HLA-typing. Chimerism analysis one month after transplant showed mixed chimerism in T-, B- and myeloid cells of both donor units and recipient. At four months after transplant the patient had 85% recipient cells in both T cells and myeloid cells indicating a threatening rejection. However, in bone marrow CD34+ cells were 90% of donor origin. Due to anticipated rejection the patient was treated with expanded CBLs, 5 × 103/kg at 4 months, 1 × 104/kg at 5 months and 1 × 105/kg at six months. Immunusuppression was tapered at six months. The immunemodulatory treatment was well tolerated with no development of GVHD. So far no change in chimeric pattern has been shown. The immunemodulatory treatment will be continued with increasing doses of CBLs.

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STANDARDIZATION OF T CELL (CD3+) DOSE FOR ALLOGENEIC PERIPHERAL BLOOD STEM CELL GRAFTS

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The number of CD3+ cells collected from donors varies widely and no significant attempts have been made to standardize the graft T cell content within the different categories of stem cell sources. Preliminary data from our center have shown that that administration of dexamethasone during the last three days of G-CSF mobilization reduces the donor T cell content of the graft by approximately 0.5 log, also resulting in a significant decrease in the incidence and severity of acute GvHD. Based on these findings, we have developed a procedure to standardize the graft to deliver a PBSC product with a T cell dose of 3.0 ± 0.5 × 10^7 CD3+ cells/kg recipient body

weight, a T cell dose comparable to bone marrow transplant, in a complete product with the exception of incidental losses that occur during the process of T cell depletion (TCD). Following collection, CD3+ T cells are enumerated by flow cytometry and a portion of the product containing a dose of 3.0×10^7 CD3+ cells/kg is set aside. The remainder of the product is depleted of T cells by a two-step method where the cells are first labeled with the anti-CD3 monoclonal antibody OKT3 attached to ferromagnetic microspheres. The product is then incubated for 30min, washed twice, and passed through a magnetized bead matrix that isolates the CD3+ T cells (CliniMACS; Miltenyi Biotec, Duarte CA). The T cell depleted product is then combined with the set aside portion of the product containing 3.0×10^7 CD3+ cells/kg for the transplant. Three separate procedures revealed 3.2 ± 0.7 log TCD for the depleted product. Recovery of other cellular components in the TCD product was as follows: B cells 75.4% ± 17.5, NK cells 33.9% ± 30.3, CD34± cells 42.0% ± 8.0. Viability of the TCD product was 97.1% ± 1.9 and sterility assessment revealed all cultures to be free of organisms following 14 days culture. Taken together, these data confirm that partial TCD can be accomplished in a closed system allowing standardization of graft T cell content.

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THE IMPORTANCE OF GRAFT CELL COMPOSITION TO OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Background: Stem cell dose has been shown to be important for outcome in patients who undergo stem cell transplantation (SCT). Grafts may contain varying amounts of different cell populations like CD3+, CD19+, CD56+, CD4+ and CD8+ immunocompetent cells, whose role for clinical outcome is less clear.

Patients and Methods: We analyzed 591 patients who underwent SCT at Karolinska University Hospital, Huddinge between 1998 to 2007 and correlated FACS results of the above mentioned cell populations to clinical outcome. Most patients (76%) had hematological malignancies. 58% of the patients received a myeloablative conditioning regimen, 62% were given peripheral blood stem cells (PBSC) and in 58% of the transplants an unrelated donor was used.

Results: As expected, PBSC contained much higher levels of all different cell populations as compared to BM. We found that patients reciving high numbers of CD4+, and CD8+ cells had significantly more rapid engraftment. Also, a CD4+ level above 200 × 10(6)/kg was correlated to higher risk of developing acute GVHD grades II-IV (41%) as compared to patients receiving below this level (24%) ($p<0{,}037$). While the incidence of bacteria sepsis was lower in patients receiving a high number of cell dose (CD3, CD19, CD56), no correlation between cell dose and CMV reactivation was found. Relapse free- and overall survivals were not significantly affected by the different cell populations.

Conclusion: Grafts containing high numbers of CD4+ cells is a risk factor for developing severe acute GVHD but survival rates were not significantly correlated to graft composition.

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HLA MISMATCHED MSC SUPPRESS T LYMPHOCYTE ALLORESPONSES IN VITRO AND DO NOT INDUCE IMMUNOLOGICAL MEMORY IN RECIPIENTS OF MSC INFUSION

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Multipotent mesenchymal stromal cells (MSC) are increasingly used to treat refractory graft-versus-host-disease and other complications in HLA-matched and mismatched hematopoietic stem cell transplantation (HSCT) patients. We evaluated immunogenicity of human allogeneic MSC infused post-transplant to HLA-mismatched, i.e. patients undergoing HSCT. We compared recipient lymphocyte response to MSC and peripheral blood lymphocytes (PBL) from the MSC or third party donors before and after infusion, and lymphocyte responses to MSC and to PBL from the MSC donor in primary and secondary challenge using ^{3}H-tymidine. MSC recipients displayed in median responses less than 500 counts per minute (CPM) to infused third party MSC 1 week to 6 months following infusion. However, the recipients responded normally to MSC donor lymphocytes, >1000 CPM ($p<0.005$), and third party lymphocytes, >5000 CPM ($p<0.005$). MSC failed to prime responder lymphocytes to rechallenge with PBL, as the responses was <10 000 versus >30 000 CPM for the corresponding control ($p<0.05$). On MSC rechallenge of lymphocytes primed with PBL from the MSC donor, only responses <500 CPM occurred. MSC upregulated lymphocyte gene expression of CD25, IFN-γ, FoxP3, CTLA-4 and IL-10 upon MSC/PBL co-culture and MSC presence in mixed lymphocyte cultures. Unprimed and primed responder lymphocytes expanded and proliferated poorly to MSC as stimuli, evaluated by flow cytometry. The MSC failed to induce CD25+ (activated) or CD57+ (effector) CD4+ or CD8+ subsets and only inconsistently induced FoxP3+ regulatory T lymphocytes. These results confirm *in vivo* and *in vitro* that infused MSC are weakly immunogenic and do not induce significant immunological memory in HLA-disparate recipients.

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DOUBLE CORD BLOOD TRANSPLANTATION (CBT) WITH EX-VIVO EXPANSION (EXP) OF ONE UNIT UTILIZING A MESENCHYMAL STROMAL CELL (MSC) PLATFORM

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Delayed or failed engraftment is a frequent complication of CBT. We developed an approach that involves ex vivo co-culture of CB mononuclear cells with third party marrow derived MSCs. Patients must have 2 CB units matched in at least 4/6 HLA antigens, with a minimum of 1×10^7 TNC/Kg per unit. A family member matched in at least 2/6 antigens or more serves as the marrow-derived MSC donor.

Methods: Diagnoses: AML/MDS (N = 4), ALL (N = 1), and CLL (N = 1). One patient was in CR and 5 had active disease (1 failed a previous CBT). Preparative regimen: myeloablative melphalan (140mg/m2), thiotepa (10mg/kg), fludarabine (160mg/m2), and rabbit ATG (3 mg/kg). GVHD prophylaxis: tacrolimus and MMF. Median weight was 57 Kg (range, 14–79). Donor-recipient HLA matching was 5 of 6 in 35% of the cases and 4 of 6 in 65%. Ex-vivo EXP: 100 ml of marrow was aspirated from the family donor and MSCs were generated in 10 T175 flasks. The CB unit with the lowest TNC dose was thawed, and placed into flasks containing confluent layers of MSCs in EXP media with SCF, FLT3, G-CSF and TPO. After 7 days at 37°C, the non-adherent cells were removed, and placed into one-liter Teflon-coated culture bags and cultured for an additional 7 days (14 days total); 50 ml of media/growth factors was added to the flasks to culture the remaining adherent layer during that time period. On day 14 the cells were washed and infused along with a 2nd unmanipulated unit.

Results: There were no toxicities attributable to EXP. The median fold EXP was 12 (1–13) for TNC and 12 (0–27) for CD34+ cells. Median time to neutrophil and platelet engraftment was 14.5 days (12–23) and 31 days (25–51). All patients (n = 6) engrafted neutrophils and platelets and became complete donor(s) chimeras. Chimerism: 1 unit dominated in all patients; 2 patients have mixed donors chimerism 4 months after CBT, with 5% and 20% of the EXP unit contributing to hemopoiesis. Three patients had no evidence of EXP unit-derived hemopoiesis after day +30, while 1 patient is 100% donor but the contributing unit