High dose therapy followed by autologous or allogeneic transplantation with peripheral blood stem cells (PBSCs) has been used to treat patients with a variety of diseases. Selection of primitive stem cells and progenitors from PBSC collections is useful for reducing the transplant volume and decreasing the number of contaminating tumor cells or T-cells. We have developed a novel approach for enumerating and enriching primitive mobilized peripheral blood cells that express high levels of the enzyme aldehyde dehydrogenase (ALDH). Mobilized cells were stained with a fluorescent ALDH substrate, termed BODIPY-aminoacetaldehyde (BAAA), and then analyzed or sorted using flow cytometry. A population of cells, termed SSCloALDHbr, was readily discriminated and comprised a mean of 3.1 ± 4.8% of the collected events. A mean of 73.4 ± 11.7% of the SSCloALDHbr population expressed CD34 and 56 ± 24.5% of all the mobilized CD34+ cells resided within the SSCloALDHbr population. The SSCloALDHbr population was largely depleted of cells with mature phenotypes and enriched for cells with immature phenotypes. The BAAA staining procedure did not diminish the viability or clonogenic activity of hematopoietic progenitors and caused no toxicity to cells or animals in a variety of pre-clinical toxicology studies. Sorted SSCloALDHbr and SSCloALDHbr CD34+ cells were enriched for progenitors with the ability to 1) generate CFUs and LTC derived CFUs, 2) expand in primary and secondary LTCs and 3) generate multiple cell lineages. In order to test whether the number of PBSC SSCloALDHbr cells would predict engraftment in actual human transplants, the total number of SSCloALDHbr cells infused per kg in 21 cancer patients who had undergone autoPBSC transplantation were compared to the times to an ANC>500 and a platelet count >20,000 using a Cox proportional hazard analysis. The time to neutrophil and platelet engraftment were both highly predictive of hematopoietic reconstitution, indicating that the SSCloALDHbr population may represent a primitive hematopoietic population that can predict engraftment following autoPBSCCT transplantation.
EX Vivo Engineering of Previously Thawed and Cryopreserved Umbilical Cord Blood (UCB) with Interleukin (IL)-2, IL-7, IL-12 and Anti-CD3 for Expansion of Cytotoxic T Lymphocytes (CTL): Promising Strategy for Adoptive Cellular Immunotherapy (ACI) Post UCB Transplantation(t)

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Limitations associated with using UCB as a source for ACI post UCBT includes the lack of donor immune effector cells from the original cryopreserved UCB unit and/or immaturity of CB cellular immunity. We have demonstrated that CTL can be selectively engineered and activated from fresh and cryopreserved and thawed (CT) aliquots of UCB (Robinson/Cairo et al, Exp Hem 30:245, 2002). In this study we evaluated and compared the activation and NK and LAK cytotoxicity of UCB CTL. Thawed UCB aliquots were monocyte depleted (5 x 10^6 cells/ml) in serum-free (SF) AIM-V in 5% CO2 at 37°C. Nonadherent cells (1 x 10^6 cells/ml) were either cultured in SF AIM-V + anti-CD3 (30 ng/ml), IL-2 (2 ng/ml), IL-7 (10 ng/ml) and IL-12 (10 ng/ml) (AB/CY) or in SF AIM-V alone for 48 hours at 37°C in 5% CO2 or expanded, recyopreserved and rethawed (TCTE), or not expanded but recyopreserved, rethawed and subsequently expanded (TCTE). NK subsets were analyzed by flow cytometry and NK and LAK cytotoxicity by WST-1 methodology using a 1:1 E:T ratio against K562 (NK) and Daudi (LAK), respectively. A significant enhancement in NK cytotoxicity was seen when UCB cells were cultured in the AB/CY cocktail (p<0.001) when compared to media alone, but no difference between the modalities (TE: 0.16±0.01 vs 0.16±0.01; TCTE:0.72±0.03 vs 0.16±0.01 and TCTE: 0.75±0.04 vs 0.16±0.01). Similarly, there was significant enhancement of LAK cytotoxicity with all modalities of AB/CY stimulation vs media alone (p<0.001) but no difference between modality (TE: 0.87±0.02 vs 0.20±0.01; TCTE: 0.37±0.008 vs 0.23±0.002; TCTE: 0.37±0.008 vs 0.23±0.002). Furthermore, there was a significant increase in the CD3+16+/56+ subset of TE, TECT and TCTE when compared to media alone (TE: 0.87±0.02 vs 0.20±0.01; TCTE: 0.37±0.008 vs 0.23±0.002; TCTE: 0.37±0.008 vs 0.23±0.002). These data suggest that previously cryopreserved and thawed UCB aliquots may be engineered at time of UCB transplant, ex vivo expanded and activated for cytotoxic (NK & LAK) potential and recyopreserved for later use for DLI post UCBT. Xenotransplant animal studies are underway to examine the in vivo effects of this UCB CTL population.

Poster Presentations - Session II