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THE ROLE OF CYTOTOXIC T CELL ANTIGEN-2 (CTLA2) IN MOUSE HEMATOPOIETIC STEM CELL (HSC) TRANSPLANT ENGRAFTMENT AND RECONSTITUTION EXAMINED BY LENTIVIRAL VECTOR TRANSDUCTION

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HSCs are rare cells resident in the bone marrow. HSCs must be capable of robust proliferation and self-renewal. The regulation of HSC proliferation occurs at least partly through interactions with the microenvironment, or niche. We found that in contrast to other bone marrow cells, HSCs constitutively express high levels of CTLA2, and that after treatment with 5-FU, CTLA2 is down-regulated in HSCs. We hypothesize that interaction between cathepsin L and the endogenous cathepsin L inhibitor CTLA2 regulates interaction of HSCs with the quiescent niche. In this study, bone marrow cells from 5-FU treated mice were transduced with lentiviral vectors encoding five different transgenes (CTLA2α and β expression constructs, siRNA CTLA2α and α/β knockdown constructs, and empty construct) and were transplanted into lethally irradiated mice with unmanipulated competitors. The short-term, long-term, and lineage engraftment of transduced HSCs was studied. Peripheral blood analysis at 4 weeks after transplant demonstrated that 90 to 100% of the animals transplanted with empty and CTLA2 knockdown constructs engrafted as oppose to 0 to 25% of the mice transplanted with CTLA2 expression transgenes. The mean percentage of peripheral blood leukocytes derived from the lentivirally transduced cells at 4 weeks post-transplant was 17% for empty construct, 19% for CTLA2α/β knokdown construct, 6% for CTLA2α knockdown construct, 1.5% for CTLA2α expression construct, and 0% for CTLA2β expression construct. The differences between empty and knockdown versus expression constructs were statistically significant. When the analysis was repeated at 8 weeks post-transplant, the same result was observed. Next, we did lineage distribution analysis of engrafted populations at >12 weeks post-transplant. We did not find significant difference between the distribution of granulocytes, T cells, and B cells within peripheral blood leukocytes derived from engrafted populations when compared to untransduced cells. Thus, our preliminary findings demonstrated that HSCs transduced with CTLA2 over expression lentiviral constructs have inferior ability to engraft (or may be impaired multilineage reconstitution?) compared to empty and CTLA2 knockdown constructs. In the future, we plan to study biological responses of modified HSCs to cytotoxic and mobilization stimuli in vivo.

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EXPANSION OF UMBILICAL CORD BLOOD DERIVED OLIGODENDROCYTES

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Transplantation of children with lysosomal storage diseases (LSD) with unrelated donor-umbilical cord blood (UCB) is effective in preventing onset and progression of severe neurologic symptoms if performed early in the course of the disease. Unfortunately, many children are not diagnosed before moderate to severe neurologic damage has occurred. These symptomatic children experience disease stabilization after transplant but do not regain lost function. This may be due to the fact that irreversible damage has occurred or, conversely, that stem cells transit to the brain too slowly to effect neural cell repair. To address this problem, we have developed in vitro methods to isolate and characterize oligodendrocytes derived from human UCB. We previously described these methods and the initial characterization of these cells (Hall et al). Continuing to advance this work, we now are focusing on further characterization and expansion of this population of cells in preparation for a phase I human clinical trial in patients with symptomatic LSD. Human UCB is red cell depleted with hetastarch, mononuclear cells are isolated with ficol density separation, and plated at a density of 5×10^5 cells/ml in media containing neurotropin 3, vascular endothelial growth factor, and platelet derived growth factor. The adherent cells are washed 2× per week for 2 weeks, then passaged and replated at a density of 5×10^3 cells/ml with media changes twice weekly, once with the original oligodendrocyte media and the second with NeuroCult media (StemCell Technologies, Vancouver). Cells expand with an approximate doubling time of 6.5 days. After 4 and 6 week periods, a 16- and 64-fold expansion can be seen, respectively. We anticipate that using a 20% fraction (200×10^6 cells) of a cord blood unit we can obtain 33×10^8 cells in 6 weeks. Oligodendrocyte lineage identity of these cells, post expansion, was confirmed by PCR based methods with expression of myelin basic protein, nestin, PLP, and Neurogenin 3. We conclude that oligodendrocytes can be isolated and expanded from human UCB yielding sufficient numbers of cells for testing in phase I human trials to facilitate neural cell repair in patients with advanced LSD.

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OPTIMUM TEMPERATURE FOR MAINTAINING THE VIABILITY OF CD34+ CELLS DURING STORAGE AND TRANSPORT OF FRESH HAEMATOPOIETIC PROGENITOR CELLS

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The optimum conditions for storage and transport of freshly harvested hemopoietic progenitor cells (HPC) in the liquid state is uncertain. It is not specified in commonly applied standards for stem cell transplantation. We used a viable CD34 assay to determine the optimum temperature for maintaining progenitor cell viability in freshly harvested bone marrow and peripheral blood stem cells. Our aim was to identify standardized conditions for storage and transport of marrow or peripheral blood products that would optimize CD34 recovery leading to better transplant outcomes. Samples were aseptically removed from 46 fresh HPC harvests (34 PBSC and 12 BM) and stored at refrigerated temperature (2°–8° C), room temperature (18°–24° C) and 37° C, for up to 72 hours. Samples were analysed for viable CD34+ cells/ml at 0, 24, 48, and 72 hours.

The mean viable CD34+ yield prior to storage was 7.7×10^6/Kg (range 0.7–30.3). The mean loss of viable CD34+ cells in HPC products at refrigerated temperature was 9.4%, 19.4%, and 28% at 24, 48, and 72 hours, respectively. In contrast, the mean loss of viable CD34+ cells at room temperature was 21.9%, 30.7%, and 43.3% at 24, 48, and 72 hours, respectively. No viable CD34+ cells remained after storage at 37° C for 24 hours. Only PBSC products and not BM showed temperature related loss of CD34 viability. Greater loss of viable CD34+ cells was observed for allogeneic PBSC compared to autologous PBSC. These results demonstrate that the optimum temperature to maintain the viability of CD34+ cells during overnight storage and transport of freshly harvested HPC is 2°–8° C. These findings allow for the development of standard guidelines for HPC storage and transport.

SUPPORTIVE CARE

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DIFFUSE ALVEOLAR HEMORRHAGE (DAH) AND INFECTION ASSOCIATED ALVEOLAR HEMORRHAGE (IAH) FOLLOWING HEMATOPOIETIC STEM-CELL TRANSPLANTATION: RELATED AND HIGH RISK CLINICAL SYNDROMES WITH POOR RESPONSE TO HIGH-DOSE CORTICOSTEROIDS

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Diffuse alveolar hemorrhage (DAH) is a non-infectious pulmonary complication of hematopoietic stem-cell transplantation (HSCT) and is associated with significant mortality. The pathogenesis and treatment of DAH is unclear. We reviewed