Growing Cord Blood Cells for Cancer Patients

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The use of umbilical cord blood (UCB) as a hematopoietic stem cell (HSC) source has been increasing in recent years and has become an important source of HSC support following myeloablative and non-myeloablative therapies. Unfortunately, it is not without restrictions. The major limiting factor to UCB application is the low cell dose available for transplantation. It is well documented that the total nucleated cell dose (TNC) transplanted per kilogram (kg) of body weight of the recipient correlates with the outcome. Patients with a total body weight of at least 45 kg who receive only a single unit of UCB have been shown to have a significant delay in time to neutrophil and platelet engraftment, as well as higher rates of engraftment failure. For this reason, UCB transplantation remains significantly more successful in children. However, even in small children who have received adequate cell doses, a delay is evident in engraftment of all cell lineages when compared to traditional stem cell sources as well as some delay with immune reconstitution.

An obvious solution to this problem would be to grow more cells to overcome the delayed engraftment and immune reconstitution. This in fact has been the goal of many studies over the past 20 or more years. Ex vivo expansion is conducted on whole UCB units as well as selected portions; these expanded products can then be infused concurrently with an unmanipulated UCB, or sometime after infusion of the unmanipulated unit. Currently, clinical protocols aimed at proving the beneficial nature of this strategy are being conducted at a number of clinical centers.

Many approaches have been explored for ex vivo expansion of UCB products from liquid culture in bags to bioreactors. A number of groups have demonstrated that selection of CD34+ cells or CD133+ cells was necessary for optimal expansion. In 1997 we reported that culture of UCB mononuclear cells (MNC) in a human growth factor (HGF) cocktail of stem cell factor (SCF) plus granulocyte colony stimulating factor (G-CSF) and thrombopoietin (TPO) resulted in only a 1.4 fold expansion of total cells, 0.8 fold in mature progenitor cells (GM-CFC) and 0.3 fold in erythroid progenitors (BFU-E). In contrast, culture of CD34+ selected UCB cells resulted in 113 fold expansions of total cells, 73 fold expansion of GM-CFC and 49 fold expansion of BFU-E. Based upon these results we initiated expansion cultures in clinical trials with CD34-selected UCB cells.

Clinical Experience:

Processing of clinical products in clinical studies has led us to two conclusions:

a) Although we can significantly expand the total nucleated cells (TNC) and committed progenitor cells from CD34+ cells, because of the significant CD34+ cell losses following the positive selection procedure, we rarely reached pre-selection TNC numbers.

b) The performance of clinical trials using UCB grafts in an unrelated setting requires the use of frozen UCB products. Selection of frozen UCB products results in significant losses of CD34+ cells (50% or greater loss of CD34+ cells) and often results in low purities. With a 50% recovery of CD34+ cells after selection we now require
at least a 400 fold cell expansion to obtain the equivalent TNC we started with. Again, from our experience with clinical studies, the purity of the CD34-selected product also impacted significantly the level of expansion achieved. The median-fold expansion obtained with products with purity greater than 50% CD34+ was 139-fold, while the median-fold expansion obtained with products with starting purities less than 50% CD34, was only 32-fold13. Therefore the use of CD34-selected products rarely results in increased cell doses of ex vivo expanded cells compared to the starting unmanipulated product. Based upon these data we have evaluated methods for expanding UCB products without an initial CD34 or CD133 selection.

Recently two groups have reported on different culture approaches for ex vivo expansion that have resulted in exciting data in clinical trials demonstrating enhanced engraftment with ex vivo expanded cells.

**Ex Vivo Expansion of Cord Blood Cells on Mesenchymal Stem Cells (MSCs)**

Based upon the ability of MSC to support hematopoietic cells, we have developed a co-culture system which is capable of expanding UCB MNC by culturing the cells on confluent MSC layers13. The literature contains many reports of the ability of MSCs to support the growth of hematopoietic cells. It has been demonstrated that MSCs produce a number of HGFs and adhesion molecules that may stimulate growth of hematopoietic cells. Our data reproducibly demonstrated a 10 to 20 fold expansion of TNC with 18 fold expansion of GM-CFC and 16 to 37 fold expansions of CD34+ cells.

At MD Anderson Cancer Center, a clinical trial is underway combining an unmanipulated UCB unit with an expanded UCB unit on a layer of MSCs. Myeloablative therapy for this protocol is ATG plus fludarabine, melphalan and thiotepa, and non-myeloablative therapy is ATG (antithymocyte globulin) plus fludarabine, cyclophosphamide and 200cGy TBI. On day 0, the unmanipulated UCB unit is infused, followed by the expanded UCB cells (from both the bags and the co-culture flasks). A median 12-fold expansion was seen in both the TNC and the CD34+ subsets. For the six recipients of myeloablative therapy, the median time to neutrophil engraftment has been 14.5 days (range 12–23) and platelet engraftment 30 days (range 25–51). Two of six patients developed Grade II Acute GvHD (graft versus host disease) which was resolved with steroids. One patient died of pneumonia in remission at day 150. Five of the six patients are alive and in complete remission at a median follow up of one year with accrual continuing14.
Notch Ligand Expansion of CB CD34+ Cells

In a variation of the liquid culture technique, Delaney et al. recently utilized an immobilized, engineered form of the Notch ligand Delta1 with recombinant cytokines (SCF, FL, IL-6, TPO and IL-3) to stimulate ex vivo UCB expansion. Ten patients with high-risk acute leukemias in morphologic remission, with a median age of 27.5 years and median weight of 61.5 kg, received myeloablative preparative regimen followed by infusion of one unmanipulated and one ex vivo expanded cord blood graft. All units were matched to the recipient at least 4/6 six loci and at least 3/6 within the average number of TNCs per kilogram. The time to ANC ≥ 500 cells/μl was shortened significantly (P = 0.002), with a median time of 16 days as opposed to a median time of 26 days (range 16–48; P = 0.002) in a concurrent cohort of 20 patients undergoing double UCB transplantation with identical conditioning and post-transplant immunosuppressive regimen.

Longer-term in vivo persistence of the expanded cell graft occurred in two subjects. In one subject, analysis at day 240 after transplant revealed that a portion (10–15%) of the donor CD14+, CD56+ and CD19+ cells were derived from the expanded graft but were no longer present by 1 year, at which point the donor engraftment was 100% from the unmanipulated cord blood graft. In the second subject, at day 180 after transplant, the contribution to engraftment from the expanded cell population in CD33+, CD14+, CD56+ and CD19+ cells ranged from 25% to 66% of total donor engraftment.

Summary

Ultimately, the goal of ex vivo expansion is the production of an optimal number of hematopoietic stem cells for graft transplantation as well as an appropriate number of specific progenitor cells for the purpose of rapid recovery from pancytopenia. A decrease in morbidity and mortality can be achieved if these goals can be met efficiently. The successful expansion with improved time to engraftment in the studies described above offer promise for studies to define the optimal cell population. The results of Delaney and colleagues demonstrate the potential of generating increased CD34+ cells, while the coculture studies on MSCs demonstrate delivery of increased number of TNCs. Future work should look at comparisons of the subsets of cells with each approach to better understand the need and contribution of these subsets to engraftment.

References

Ion McNiece obtained his PhD from Melbourne University in 1986 and his thesis work was undertaken at the Peter MacCallum Cancer Institute under the direction of Drs Kriegler and Bradley. He did a post doctoral fellowship with Dr Peter Quesenberry in Charlottesville, Virginia and then worked at Amgen in California for 9 years studying hematopoietic growth factors. Dr McNiece returned to academia and worked at the University of Colorado in Denver as Director of Research in the Bone Marrow Transplant Program. He then moved to Johns Hopkins University as Professor of Oncology and Director of the Graft Engineering Laboratory. In 2004 he was appointed new director of the division of Biomedical Sciences of Johns Hopkins in Singapore. In 2007 he moved to the University of Miami where he was a Professor of Medicine and Director of the Experimental and Clinical Cell Based Therapies Program in the Stem Cell Institute. His work has focused on translational approaches using hematopoietic stem cells and the generation of cellular products for therapeutic approaches. In July of 2012 he moved to MD Anderson in Houston, Texas where he is the Director of the Cellular Therapy Laboratories where he oversees the clinical production of cellular products for clinical trials and standard of care procedures. His research laboratory is focused on developing culture conditions for optimal generation of cellular products for cardiac and hematopoietic therapies.


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