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Ex vivo expansion of umbilical cord blood

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The efficacy of cord blood (CB) transplantation is limited by the low cell dose available. Low cell doses at transplant are correlated with delayed engraftment, prolonged neutropenia and thrombocytopenia and elevated risk of graft failure. To potentially improve the efficacy of CB transplantation, approaches have been taken to increase the cell dose available. One approach is the transplantation of multiple cord units, another the use of ex vivo *expansion. Evidence for a functional and phenotypic heterogeneity exists within the HSC population and one concern associated with* ex vivo *expansion is that the expansion of lower 'quality' hematopoietic progenitor cells (HPC) occurs at the expense of higher 'quality' HPC, thereby impacting the reserve of the graft. There is evidence that this is a valid concern while other evidence suggests that higher quality HPC are preserved and not exhausted. Currently,* ex vivo *expansion processes include: (1) liquid expansion: $CD34^+$ or $CD133^+$ cells are selected and cultured in medium containing factors targeting the proliferation and self-renewal of primitive hematopoietic progenitors; (2) co-culture expansion: unmanipulated CB cells are cultured with stromal components of the hematopoietic microenvironment, specifically mesenchymal stem cells (MSC), in medium containing growth factors; and (3) continuous perfusion: CB HPC are cultured with growth factors in 'bioreactors' rather than in static cultures. These approaches are discussed. Ultimately, the goal of* ex vivo *expansion is to increase the available dose of the CB cells responsible for successful engraftment, thereby reducing the time to engraftment and reducing the risk of graft failure.*

Keywords

Cord blood, ex vivo *expansion, mesenchymal stem cells (MSC), liquid culture, MSC co-culture, continuous perfusion.*

Introduction

Umbilical cord blood (UCB) has become an important source of hematopoietic stem cell (HSC) support following myeloablative and non-myeloablative therapies [1–6]. In addition to increasing the HSC donor pool for the general population, cord blood (CB) is playing an increasingly important role in improving the donor HSC pool for minority populations currently poorly represented in donor registries.

One major drawback that currently limits the efficacy of CB in HSC therapy is the low cell dose available for transplantation. A correlation between the number of CB mononuclear cells (MNC) transplanted per kilogram body weight and time to engraftment suggests that patients >45 kg receiving a single CB unit will have a markedly prolonged time to engraftment (as measured by neutropenia and thrombocytopenia) and higher rates of engraftment failure [7–11]. As a consequence, the majority of patients receiving successful CB transplantation to date have been children (average weight 20 kg) [9,10,12]

Two approaches have been taken to increase the total number of CB MNC transplanted, thereby improving the feasibility of CB transplantation for patients >45 kg. One has been to increase the total number of CB MNC transplanted by the transplantation of multiple cords [13–17]. The second has been the use of *ex vivo* CB expansion. *Ex vivo* expansion can be performed on whole CB units prior to transplantation, or a fraction of a CB unit that is recombined with its 'unmanipulated' fraction at the time of transplantation, or transplanted a period of time after the unmanipulated fraction. Indeed, the combination of *ex vivo*-expanded and unmanipulated CB fractions might prove a beneficial strategy [18,19]. Clinical protocols that explore these approaches have been, and are currently being, assessed at the University of Texas MD

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Anderson Cancer Center (Houston, TX, USA) and in other clinical centers [19–21].

Although discussed specifically with respect to CB HSC, *ex vivo* expansion strategies can also be applied to BM- and peripheral blood-derived HSC [22–24]. There is evidence of functional and phenotypic heterogeneity within the HSC population [25–29] and one concern associated with any *ex vivo* HSC expansion strategy is that short-term reconstituting lower 'quality' HSC are expanded at the expense of long-term reconstituting higher 'quality' HSC, thereby significantly impacting the HSC reserve of the graft, in effect 'robbing Peter, to pay Paul' [30]. There is evidence, primarily in animal models, that suggests that this may occur under certain conditions. For example, McNiece *et al*. report compromised long-term repopulating activity following *ex vivo* expansion in a fetal sheep model [22,31]. Von Drygalski *et al*. report the loss of radioprotective and long-term engraftment potential with *ex vivo* expansion of murine bone marrow [32], and in a clinical study Holyoake *et al*. report the absence of durable engraftment from *ex vivo*-expanded $CD34^+$ cells [33]. There is also evidence to the contrary. Piacibello *et al*. observed evidence of self-renewal and amplification of HSC during *ex vivo* expansion [34], Lewis *et al.* report that CB cells capable of engraftment in primary, secondary and tertiary xenogeneic recipients are preserved following *ex vivo* expansion [35], and Guenechea *et al*. report a delay in engraftment in a mouse model, suggesting that potentially more primitive, less rapidly engrafting cells are preserved during *ex vivo* expansion [36]. While these issues remain to be clarified, there is evidence that short-term *ex vivo* HSC expansion does not impact the homing of the HSC, an important consideration for their use in transplantation [37].

While *ex vivo*-expanded products may possess an inherent reduction in long-term hematopoietic reconstitution potential under certain conditions [30,31,33], this potential skewing of the CB product to a more rapidly reconstituting but lower 'quality' HSC profile may be used to clinical advantage, especially when *ex vivo*-expanded and 'unmanipulated' fractions of the same CB, or different *ex vivo*-expanded and 'unmanipulated' CB samples, are combined for transplantation. Clinical data suggest that the *ex vivo*-expanded CB fraction provides a more rapid (albeit short-term) hematopoietic reconstitution, while the 'unmanipulated' CB sample provides the less rapid (albeit long-term and sustained) hematopoietic reconstitution [19]. Conversely, other clinical data suggest that there is no significant benefit to augmenting transplanted, unmanipulated CB cells with *ex vivo*-expanded cells [19–21].

The goals of *ex-vivo* expansion are two-fold. While a major focus is directed at providing optimal numbers of HSC for transplantation, the generation and transplantation of more lineage-committed hematopoietic progenitors to abrogate chemotherapy-induced pancytopenia is also an important goal. Currently, there are several different strategies used for *ex vivo* expansion.

Liquid culture

A number of groups have chosen to expand CB in liquid culture, in an attempt to increase the number of committed and/or primitive CB progenitors available for transplantation. Liquid culture expansion requires that primitive hematopoietic progenitor cells (primarily $CD133^+$ or $CD34^+$) first be isolated from fresh, or frozen, hematopoietic tissue (CB, BM or growth factor-mobilized blood) [24]. Clinically, a number of techniques are available to perform this isolation, including the Miltenyi MACS® system (Biotec, Inc., Auburn, CA, USA) or the Baxter Isolex® device (Baxter Deerfield, IL, USA) and purities of greater than 90% $CD133^+$ or $CD34^+$ can be achieved. Isolated $CD133^+$ or $CD34^+$ cells are subsequently incubated in culture medium, in some cases containing FBS and supplemented with a cocktail of growth factors targeted at stimulating the proliferation of primitive hematopoietic progenitors. Components of the growth factor cocktails used in *ex vivo* HSC expansion protocols include: SCF, IL-3, IL-6 and G-CSF [24]; SCF, thrombopoietin (TPO) and G-CSF [18,23]; Flt-3 ligand (FL), SCF, IL-3, IL-6 and G-CSF [38,39]. FL and TPO appear to be important in supporting the self-renewal of primitive stem cells, possibly by preventing telomere degradation with proliferation [38,40], while SCF and IL-6 possibly enhance the proliferative potential of specific HSC subpopulations [41–44]. IL-11 has also been incorporated into *ex vivo* expansion cocktails [32,45–49].

In a 37-patient study (25 adults and 12 children, 34 patients with hematologic malignancies and three patients with breast cancer), Shpall *et al.* [18] demonstrated the efficacy of *ex vivo* expansion of isolated $CD34^+$ CB cells. HLA-matched, or closely matched, CB units were identified (matched at 6/6 for nine patients, 5/6 for 22 patients and 4/6 for six patients). In the majority of cases the CB was frozen in two fractions (40/60%). Prior to CB