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Semin Hematol. Author manuscript; available in PMC 2012 March 26.

Published in final edited form as:
Semin Hematol. 2010 January ; 47(1): 97–103. doi:10.1053/j.seminhematol.2009.10.002.

Umbilical Cord Transplantation: Epilogue

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Introduction

Conferences have recently been held to celebrate the success of the first cord blood (CB) transplant.[1] It has been more than 20 years since publication of the laboratory studies[2,3] and first clinical CB transplant[4] that initiated the field of CB transplantation (T) as a viable alternative to bone marrow (BM) T. Events leading to the laboratory efforts and first CB transplant have been reviewed elsewhere.[4-7] The first CBT utilized CB cells from an HLA-matched female sibling donor to treat her brother,[3] a treatment that cured the disorder and likely eventual fatal hematological manifestations of Fanconi anemia. The recipient is presently alive and well. CBT has since been used to treat a wide variety of malignant and non-malignant disorders using completely HLA-matched or partially disparate HLA allogeneic cells for children and adults. More recent selected studies have documented successes in leukemia/cancer,[8-12] Fanconi anemia,[13] severe aplastic anemia,[14] myelodysplastic syndrome,[15] and other disorders.[16] In addition to CBT in myeloablated recipients, it has also been used after non-myeloablative conditioning.[17,18] Information on outcomes of CB transplants have been summarized in recent review articles.[7,19] Advances in the treatment of adults with CBT has involved use of larger numbers of donor cells for transplantion through use of cells from 2 donor cell collections.[7,19-21] CBT has also been used autologously for a child with leukemia.[22] Because of the successes of CBT, it is easy to forget the initial fears for clinical CBT (reviewed in [5-7]). These included serious concerns as to whether single collections of CB contained hematopoietic stem cells (HSCs), if there were enough HSCs and hematopoietic progenitor cells (HPCs) to allow short- or long-term engraftment with normal healthy cells, whether there might be enhanced graft vs. host disease (GVHD) elicited by donor CB cells due to maternal cell contamination of the collected CB, and if CBT could be used to treat the wide variety of disorders treated by BMT, including leukemia and other cancers, where enhanced relapse was a concern when it became clear that CBT elicited less, not more, GVHD than BMT. These concerns have been largely allayed, but further scientific and clinical advances will be required for the full potential of CBT to be realized.

The lifetime probability of undergoing a hematopoietic stem cell (HSC) transplant is much higher than previously reported.[23] It was noted that this probability could rise higher with increases in donor availability and applicability of transplants.[23] The first “proof-of-principle” CB bank, which supplied the first 5, as well as two of the next 5, HLA-matched

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sibling donor CB units for transplantation, was set up in the author's laboratory. These first CB transplants demonstrated the feasibility and reality of CBT, and rigorously proved that CB contained clinically applicable numbers of long-term engrafting HSCs.

The feasibility of performing unrelated CBTs, which encompass the overwhelming majority of CBTs done to date, was made possible by establishment of CB banks storing HLA-typed allogeneic CB units. This past year is the first in which the number of CB transplants outnumbered the number of BM transplants performed.[1] With the increasing number of CB banks that maintain higher numbers of quality CB units, and the enhancement of numbers of such units also obtained from donors from ethnically-diverse backgrounds, a better understanding of the biology of HSCs, and the knowledge to manipulate CB HSCs for clinical efficacy, there will likely be large increases yearly in the number of CB transplants done. This will enhance the lifetime probability of undergoing an HSC transplant to correct life-threatening health disorders.

The above information reviewed in brief where the field of CBT has been. The remainder of the epilogue focuses on where we are going, or need to go, to effectively advance the field of CBT. This includes the search for potential means to improve engraftment of HSCs, especially those present in CB, an event that requires a better understanding of the extrinsic and intrinsic factors that mediate HSC function. It also includes relatively new technologies and information that suggest potential additional uses of CB as a treatment modality.

Hematopoietic Stem Cells

HSCs in mice are well characterized phenotypically and functionally. Originally defined phenotypically as a population of Sca1^{+}c-kit^{+} lineage^{-} (SKL) cells, further refinements placed mouse HSCs into a CD34^{-} SKL or CD150^{+}CD48^{-}SKL population.[24,25] Human HSCs are not as well defined as those in the mouse system, and are found in a CD34^{+}CD38^{-} population of cells; HPCs segregate mainly into a CD34^{+}CD38^{+} population. In order to better define potency of a CB unit, it would be useful to be able to better phenotype human HSCs. Phenotype does not always recapitulate function, especially when cells are manipulated ex-vivo. Thus, it is important that phenotype is a measure of function. Human CB HSCs have been reported to be highly enriched in a CD34^{+}CD38^{-}lineage^{-} CD90^{+}CD45RA^{-} population, while candidate human multipotent progenitors are found in a CD34^{+}CD38^{-}lineage^{-}CD90^{-}CD45RA^{-} population.[24] The HSC population in this study[24] was functionally defined by the ability of this purified population to engraft Non Obese Diabetic (NOD) severe combined immunodeficiency (SCID) mice with a NOD.Cg-Prkdc^{SCID}IL2rg^{tmWjl}/$_{SzJ}$ (NOG) genotype. Phenotypes of mouse and human HSCs, multipotent progenitors, common myeloid progenitors, megakaryocyte erythroid progenitors, and granulocyte macrophage progenitors have been reviewed.[25] While hematopoietic cell development was considered a catenated pipeline from HSCs to HPCs to precursors to mature blood cells, it is apparent that this model is not absolute as mature cells can now, under the right conditions be induced into a pluripotent state. The concept of induced pluripotent stem (iPS) cells will be covered at the end of the epilogue, along with the potential significance of iPS cells to enhanced utility of cord blood. HSCs/HPCs have been characterized to limited degrees by genomics and proteomics (e.g. see [26,27]). Recent studies suggest the possibility of enhanced insight into HSCs and HPCs through imaging hematopoietic precursor division in real time,[28] visualizing spatiotemporal dynamics of multicellular cell-cycle progression,[29] profiling by high-throughput sequencing of the transcriptional activities of cells,[30] probing mitotic history and developmental stage of hematopoietic cells using single telomere length analysis,[31] and analyzing the intracellular signaling activity of HSCs and HPCs at a single cell level.[32] Asymmetric and symmetric cell divisions are a hallmark of HSCs, and are used as models of the self-renewal of HSCs and production of HPCs. HSC numbers can be enhanced when both daughter cells become

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HSCs, maintained when only one daughter cell becomes an HSC and the other becomes an HPC, and decreased/lost when both daughter cells are HPCs. Asymmetric segregation of organelles or proteins[33] likely plays an important role in determining which daughter cells become an HSC and which an HPC.[34] Mitochondria are central effectors of cells that generate cellular energy. Careful analysis of segregation of mitochondria during cell division may be informative in defining which daughter cells become an HSC versus an HPC.

A key to the viability of CB banking is the capacity to cryopreserve HSCs and HPCs in CB, and therefore maintain them long-term in a frozen state with subsequent efficient recovery of viable and functional cells after long-term storage. The longest storage time reported with efficiency recovery of functional HSCs and HPSs from cord blood is 15 years,[35] but we have recently determined that HSCs and HPCs from CB can be maintained in stored cryopreserved form for at least 23 years (Broxmeyer, unpublished observations).

Means to Enhance the Applicability of CB cells for Clinical Utility

In addition to double CB transplantation,[7,19-21] there are other potential means to enhance numbers and/or functional capacities of HSCs and HPCs in cord blood. This includes: i) obtaining greater numbers of cells from the cord blood and placental blood vessels, ii) enhancing homing and engrafting capabilities of obtained CB HSCs, and iii) expanding CB HSCs ex-vivo, and/or in vivo.

i) Obtaining Increased Numbers of HSCs and HPCs from CB/Placental Vessels—Our original efforts to collect CB cells for clinical transplantation focused on obtaining as many cells as possible from the cord itself,[2,3] efforts that depended largely on the skills of those doing the collecting. After collection of as much blood as possible, additional cells were collected from placental blood vessels. The combination of efforts allowed retrieval of maximal numbers of nucleated blood cells. Studies of others subsequently evaluated use of perfusing the placenta, which enhanced numbers of CB HPCs collected.[36] Most recently, we found that perfusion of placental vessels, after maximal collection from the cord itself, resulted in collection from the placenta of as many cells as those collected from the cord, essentially doubling the collection of HPCs.[37] Placental perfusions are not easy to perform as every placenta is different, and such perfusions require skill and technical expertise. Thus, routine use of placental perfusions is not likely to occur in the near future, and when it does it will likely be tested in a limited number of specified collection centers. HSC development occurs in the placenta itself.[38,39] Thus, it may be possible to use HSC/HPC extraction/ isolation from placenta to complement collections of HSCs and HPCs from cord blood and after perfusion of placental blood vessels. A main concern with collecting cells from placental vessels or from the placenta itself is the possibility of maternal cell contamination which could present a potential problem of the cells eliciting increased levels of GVHD. If collections of cells from placental vessels by perfusion, or from placenta, do not result in greatly increased numbers of maternal cells being collected, it is suggested by the author that serious consideration be given to also collecting, freezing, and storing placental cells for potential clinical transplantation.

ii. Enhancing Homing and Engrafting Capabilities of CB HSCs—Engraftment requires that donor HSCs migrate to the bone marrow, site of production in children and adults of HSCs, HPCs and other blood cell types, where cells are nurtured for self-renewal, proliferation, survival, and differentiation. Towards this end, it is important to better understand recipient bone marrow microenvironmental niches for HSCs and HPCs, as well as characteristics of HSCs and HPCs themselves. There has been an enhanced understanding of these HSC/HPC niches.[40-42] In brief, microenvironmental niches contain a number of

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different stromal cell populations including osteoblasts, osteoclasts, endothelial cells, fibroblasts, and mesenchymal stem/stromal cells (MSC). How and in what way these cells may interact amongst themselves, and interact also in the context of donor HSCs and HPCs, is far from clear, but these interactions require a number of different cytokines, receptors, and adhesion molecules, and may involve the nervous and immune systems.

A number of laboratories have evaluated means to enhance homing/engraftment through ex-vivo manipulation of donor HSCs/HPCs, and/or the "accessory" cells present in the donor cell inoculum. We evaluated inhibition of CD26/Dipeptidylpeptidase IV as a means to enhance homing/engraftment, and found that a single ex-vivo exposure of donor cells to either of two peptides (Diprotin A = Ile-Pro-Ile; Val-Pyr) enhances mouse bone marrow cell engraftment of lethally irradiated recipient mice,[43] and human CB $CD34^+$ cell engraftment of NOD/SCID mice.[44] Other groups have also seen similar enhanced engraftment after inhibition of CD26.[45-49] Others have demonstrated that exposure of donor cells to a stable analogue of Prostaglandin-E1 (PGE1) greatly enhances engraftment of mouse bone marrow cells into lethally irradiated mouse recipients.[50,51] How PGE1 works is not known,[52] although hints as to this mechanism have been reported.[51] Investigators reported that CB $CD34^+$ cells treated with GDP fucose and α1-3fucosyltransferase-VI were increased in their capacity to engraft NOD/SCID mice.[53] Activation of guanine-nucleotide-binding protein stimulatory alpha subunit ($G_{\alpha}s$) enhances homing and engraftment of mouse HSCs[54]. Some of the above procedures may turn out to be of clinical value for enhancing engraftment of human HSCs. Since mechanisms of action of these reagents seem to be different, combinations of these procedures may enhance engraftment even further. While most of these procedures enhance engraftment, it will be important to determine if they also enhance speed/time to engraftment, an event of particular concern for CB where time to neutrophil and platelet recovery is delayed compared to BM and mobilized peripheral blood cells.[3,7]

One possible way to "bypass" the need for enhancing homing is direct injection of donor cells into bone marrow spaces (termed intra BMT). Efforts in this area are ongoing using injection of donor mouse and human bone marrow cells into mice,[55] and also in the context of transplantation into non-human primates.[56,57] Clinical studies in this area are also ongoing. It remains to be seen whether intra marrow injection of donor cells proves to be a significant means of enhancing engraftment or time to engraftment, and whether the above means of treating the cells with agents that enhance intravenous engraftment can further increase the engrafting capability of intra marrow transplanted cells.

ii) Expanding CB HSCs Ex-Vivo, and/or In-Vivo—A means to enhance engraftment would be to expand ex-vivo or in-vivo donor HSCs and HPCs. To reach this goal, which has not yet been realized in the context of human HSC transplantation, it will be necessary to better understand the biology of HSCs, their interactions with growth promoting cytokines, the intracellular signals that mediate HSC self-renewal, proliferation, survival, and differentiation, and the intimate relationship between HSCs and their marrow microenvironmental niches. Recent in-depth reviews describe many, but not all, of the cytokines, receptors, and intracellular signaling molecules involved in regulating the functional activities of HSCs/HPCs, and hematopoiesis.[58,59] Understanding the network of interacting cell cycle and transcriptional factor regulatory genes/proteins, and better insight into the process of stem cell self-renewal, should provide us with future means to best modulate HSC functions for clinical utility. Transcriptor factors are key to HSC functions.[58,59] The author believes that much can be learned about HSC renewal and production by studying embryonic stem cells (ESCs), since ESC lines have extensive self-renewal capacity in vitro. SIRT1, a member of the Sirtuin family of deacetylases, which has effects on various biological systems,[60] has been shown,[61] along with other factors, to be important in maintenance of ESCs in vitro. We have evidence that SIRT1 is important for

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mouse fetal liver and adult bone marrow HPC proliferation in vivo, especially in context of lowered oxygen tension (Broxmeyer, unpublished observations) and SIRT1 may be important for maintenance and/or expansion of HSCs. The Ras subfamily member Rheb (ras homologue enriched in brain) 2 has been linked to HSC/HPC production. There are increases in HPC and early engraftment of short-term repopulating cells in Rheb2 overexpressing cells, but this is at the expense of the long-term repopulating HSCs. Modulation of intracellular signals such as Rheb2 may possibly be of clinical value. CD26 not only truncates the chemokine SDF-1/CXCL12, a reason why inhibition of CD26 enhances homing and engraftment,[43-49] it also truncates other cytokines such that mice that are CD26 null recover more effectively from cytotoxic stresses due to drugs (e.g. Ara-C and 5-FU) and irradiation (Broxmeyer, unpublished).

Other Potential Uses of Cord Blood

Mesenchymal stem/stromal cells (MSCs) have extensive proliferative capacity in vitro and can be induced under appropriate culture conditions to differentiate into multiple lineages, including bone, fat, and cartilage.[63] MSCs have a number of attributes, such as being immunomodulatory, that suggest their potential for clinical applicability;[64] however, there is much to be learned in this area.[65] MSCs can be obtained from BM and expanded, but there is much more variability in presence or numbers of these cells in CB than BM.[7] While CB MSCs may present an additional CB cell type for future clinical use, investigators will have to deal with the lowered numbers of CB units that may contain MSCs, perhaps because of the lowered frequency of these cells compared with those obtained from BM. CB is also a source of endothelial progenitor cells (EPCs) with extensive proliferative capacity,[66] that have the in vivo potential to form functional long-lasting vessels.[67] There is an ongoing controversy in terms of defining EPCs and what their role is in angiogenesis, at least in the mouse system.[68] Use of EPCs and other endothelial cells in the clinic have been reviewed.[69]

It is also possible to expand numbers of mature blood cell types, such as erythrocytes[70,71] and platelets[72] from CB HPC. Whether the numbers of these mature cells will be enough, or can be further increased, for therapeutic advantage is not yet determined.

Perhaps the most exciting advance in stem cell biology is the development of the emerging field of induced pluripotent stem (iPS) cells, first reported for mouse,[73,74] and subsequently for human cells.[75,76] Through the use of enforced expression of a number of transcription factors such as Oct4, Sox2, KLF4, and/or c-myc or other factors, mature cell types can be reprogrammed to an ESC-like state, and these cells can be subsequently differentiated down a number of different cell/tissue lineages. These iPS cells can be generated from human blood,[77] and likely from CB. Disease specific iPS cells have been generated,[78] and mice with a humanized sickle cell anemia have been rescued from the disease after being transplanted with HPCs obtained from autologous iPS cells generated in vitro.[79] The original iPS cells were generated by retroviral induced transcription factors, which presents the potential for insertional mutagenesis. Transduction of c-myc offers the possibility for cellular transformation of the transduced cells. A number of technologies are currently being used to counter the possibilities of insertional mutagenesis and transformation by using non-integrating plasmid delivery of the transcription factors or delivery or induction of the transcription proteins themselves. Time will likely yield the most efficient and safest means to produce iPS cells, but not all iPS cells are created equally; iPS cell clones and their sub-clones may not all be at the same level of reprogramming to early cell states or have the same capacity for differentiation.[80] As the iPS field advances, so will the potential to generate cells from CB that may have the capacity to be used in a regenerative medicine sense.

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Concluding Thoughts

The field of CBT has come a long-way since the first laboratory studies that suggested,[2] and the first CB transplants[3-7] that proved, the clinical efficacy of these cells. Each new advance in our understanding of HSCs in general and CB HSCs in particular, and in other possible uses of CB cells as noted above, enhances the potential for increased clinical use of CB cells. This in turn highlights the need for greatly increased numbers of CB banks and expansion of the inventory of HLA-typed high cell number and quality CB units. We are in an exciting time in the area of CB biology and transplantation, and I look forward to future advances that will make CB an even more useful product.

Acknowledgments

Some studies referred to in this article from the author's laboratory were supported by US Public Service Health Awards: R01HL5646, R01HL67384, and a Project in P01HL67384 from the National Institute of Health to HEB.

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