

Insights into the biology of cord blood stem/progenitor cells

H. E. Broxmeyer

Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN, USA

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Abstract

Objectives: To review information on cord blood banking and transplantation with respect to the author's studies, and in context of this field of investigation.

Results: Cord blood transplantation has been successfully used to treat a number of malignant and non-malignant disorders. However, this technique is still associated with limited numbers of cells for transplantation, and with delayed engraftment of neutrophils and platelets. The field of cord blood transplantation will benefit from enhanced and mechanistically based information on haematopoietic stem cell function and potential means to enhance its effectiveness are reviewed. This includes notions concerning possibility of retrieving more cells from the placenta and cord blood, to expand haematopoietic stem cells *ex vivo* and to increase efficiency of homing and engraftment of these cells. Also discussed are cryopreservation and long-term storage of cord blood haematopoietic and progenitor cells, and new laboratory findings and animal studies for non-haematopoietic uses of cord blood.

Introduction

The first ever cord blood transplantation was performed at the Hôpital St. Louis, Paris, France in October, 1988; this transplant used HLA-matched sibling cord blood cells from a sister to treat her young brother with Fanconi anaemia (1). The recipient was cured of haematological manifestations of Fanconi anaemia and is currently alive and

well almost 22 years since the procedure. This first transplant was initiated based on extensive laboratory investigations (2,3) as well as on some proof-of-principle studies using neonatal blood from mice, to reconstitute lethally irradiated ones (3). Some of the background leading up to laboratory studies and initial HLA-matched sibling cord blood transplantation had also been reported (1–7). Since those initial clinical studies, made possible in part by the proof-of-principle cord blood bank set up in the author's laboratory, there have been numerous public and family banks set up world-wide, with the public banks contributing HLA-typed cord blood units for over 20 000 allogeneic cord blood transplants performed to date (8). These procedures have treated an array of patients with numerous malignant and non-malignant disorders, similar to those treated with bone marrow transplantation (6).

Noted amongst advantages of cord blood units for use of haematopoietic stem cells contained within it to allow recovery of the blood cell system, are ease of collection, ready availability when stored in a cryopreserved state, and relatively low graft-versus-host disease elicited by cord blood cells compared to bone marrow cells (6). However, there are disadvantages in the use of cord blood. Such include limited numbers of cells obtained in single cord blood collections, which, while fine for transplantation in children and low weight adults, is less effective in full-size adults and high weight children. To compensate for this, double cord blood units have been used for transplantation in adults (9–12). There is presently no rigorous proof that two cord bloods are better than one, but it allows patients who might otherwise be excluded from receiving a cord blood transplant (due to lack of available adequate-sized single cord blood unit), to receive cord blood transplantation. In most cases, only one of the two cord blood collections actually engraft long-term, and double cord blood unit transplants have been associated with greater levels of graft-versus-host disease, than that seen with single cord blood transplants. Another disadvantage of cord blood transplantation, whether one or two cord blood units are used, is the slower time for neutrophil and platelet engraftment. These disadvantages of cord

Correspondence: H. E. Broxmeyer, PhD, Department of Microbiology and Immunology, Indiana University School of Medicine, 950 West Walnut Street, R2-302, Indianapolis, IN 46202-5181, USA. Tel.: 317-274-7510; Fax: 317-274-7592; E-mail: hbroxmey@iupui.edu
Dr Broxmeyer is on the Medical Scientific Advisory Board of the Public and Family Cord Blood Banking Company, CORD:USE.

blood for sustaining blood cell replacement highlight the need for innovations to enhance efficacy of cord blood transplantation; innovations will derive from increased laboratory and clinical investigation. Several possibilities for this exist, including: removal of more haematopoietic stem and progenitor cells from placental and cord blood, enhancing *ex vivo* expansion of haematopoietic stem and progenitor cells, and/or enhancing homing and engrafting capabilities of the cells.

Potential means to enhance effectiveness of cord blood transplantation

Obtaining more cells from placental and cord blood. Our original efforts in this area were instituted during collection of cord blood for the first and next six HLA-matched sibling cord blood transplantations, in which as much cord blood as possible was collected from the cord, and then a syringe being used to collect blood from placental vessels (2,3). Cord blood frozen and stored, then used for the first five and then following two of the next five cord blood transplants, were a combination of total amount of cord and placental blood collected. More recently, a number of efforts have been made including by our own group (13) to perfuse the placenta after as much cord blood as possible was first collected. It is apparent that one can acquire more blood after perfusion of the placenta than that retrieved by draining the cord only; however, with perfusion comes the danger of also collecting maternal cells. Maternal cell contamination would need to be rigorously checked, as excess maternal cell contamination has the potential to elicit higher levels of graft-versus-host disease. Efforts to enhance output of cells during perfusion of the placenta using perfusion material that might mobilize early blood cell types, such as AMD3100 [known to enhance mobilization from adult peripheral blood (14)], would also require extensive controls with which to compare cell collections. The problem is how to prove that addition of a 'mobilizing' agent to the perfusate, actually increases cell output. Because of great variability in numbers of cells collected between cord blood units from different babies, one must use the same placenta and cord as internal control to show that after perfusion with control medium has extensively exhausted further release of cells, the test material can further enhance this output. To the author's knowledge, no one has adequately proven that this is the case. In a further context, a number of groups have demonstrated that the placenta itself, and not placental blood vessels, is a rich source of haematopoietic stem and progenitor cells (15–18). This opens up the possibility that after collection of blood from the cord and placental vessels, the placenta may serve as an additional source of the cells. Here also,

one would have to ensure that there is a very low level of maternal cell contamination in the placenta-derived cells.

Ex vivo expansion of haematopoietic stem cells. Investigators have been trying for many years to expand human haematopoietic stem cells *ex vivo* (19). This has been accomplished for mouse haematopoietic stem cells and for human and mouse haematopoietic progenitor cells, but no one has yet adequately demonstrated *ex vivo* expansion of long-term engrafting human haematopoietic stem cells. Efforts in this area have now focused on enhancing the environment *in vitro* needed to nurture survival, proliferation and self-renewal of the stem cells, including use of potent combinations of cytokines and growth factors; however, clinical efforts in this arena have not yet been very encouraging (6). A potentially promising clinical effort recently reported has suggested that use of Notch ligand-mediated expansion allowed for more rapid reconstitution of myeloid cells in the context of double unit cord blood transplantation (20). Evidence though, that Notch mediated *ex vivo* expansion of a long-term marrow engrafting haematopoietic stem cell, was not provided. It is clear that we need a better basic understanding of stem cells, cells in the microenvironment, and their interactions that regulate haematopoietic stem cell function, if we are to be able to truly expand haematopoietic stem cells *ex vivo*. A more mechanistic comprehension of receptors and intracellular network of signalling molecules – activated or repressed by microenvironmental cells and cytokines – will be crucial to such an undertaking. The list of potential intracellular mediators includes, but is not limited to: Notch ligands/Notch, Wnt3a, HoxB4/PBX1, Bmi1, c/EBP alpha, Gfi-1, p21^{cip1/waf1}/p27^{kip1}, PTEN, NOV/CCN3, PUMA, glycogen synthase kinase-3, p38 MAP Kinase, nucleophosmin, MEF/ELF4, STAT3, STAT5, Mad2, Rheb2, M-TOR and Sirt1 (reviewed in part in: 19). It is possible that once we know which receptors and intracellular signals are selectively involved in enhancing haematopoietic stem cell functions for expansion, we may be able to bypass the need for stromal cell interactions of the niche, and specific cytokines or combinations of cytokines. Instead, we may be able to use pharmacological agents to elicit comparable *ex vivo* expanding effects. For example, we recently reported that overexpression of Rheb2 enhances mouse haematopoietic progenitor cell population growth *in vitro* and short-term repopulation *in vivo*, the latter in a transient fashion. However, this comes at the expense of long-term haematopoietic repopulation *in vivo* (21). Rheb is a member of the Ras homologue, enriched in brain family of small Ras-like GTPase molecules, which cycle between active GTP and inactive GDP-bound forms. Both Rheb1 and Rheb2 are able to activate signalling of mammalian target of rapamycin

(mTOR), in mammalian cells. These effects of overexpression of Rheb2, using a MIEG bicistronic retroviral vector in which cloned Rheb2 had a flag-tag at the N-terminus of the protein, and which also had a reporter gene for enhanced green fluorescence protein, were found to be sensitive to rapamycin treatment. Thus, the means to modulate this signalling pathway that incorporates rapamycin-sensitive mTOR pathway, may in the presence of cytokines, enhance functional capacity of and/or expand, human haematopoietic stem cells *in vitro*. We are currently evaluating this possibility using *ex vivo* cultures of human cord blood CD34⁺ cells, with functional read-out of human haematopoietic stem cell engraftment, in mice with non-obese diabetic severe combined immunodeficiency (NOD/SCID), and an IL-2 receptor gamma chain knock-out. We have also been working on modulation of the sirtuin family member, Sirt1, as a means to enhance mouse and human haematopoietic stem cell function, based on our recent studies demonstrating that Sirt1 is involved in maintenance of mouse embryonic stem cell population growth, specially under stress from and response to reactive oxygen species (22). This activity involves Nanog expression, and translocation of tumour suppressor gene protein p53 between the nucleus and mitochondria (22). In this context, it is likely that a better understanding of a role for mitochondrial number and activity, and their modulation in haematopoietic stem cells (23), may allow us a means to enhance human haematopoietic stem cell function.

Enhancing homing and engraftment capabilities of haematopoietic stem cells through inhibition of CD26/dipeptidylpeptidase IV enzymatic activity. Engraftment of haematopoietic stem cells encompasses at least two separate but interrelated events, including homing of cells to the bone marrow, and then nurturing them for survival, proliferation, self-renewal and differentiation, within the marrow microenvironment. A ligand-receptor interaction strongly implicated in homing and engraftment of haematopoietic stem and progenitor cells is that, respectively, of stromal cell-derived factor-1 (SDF-1/CXCL12) and CXCR4 (24,25). SDF-1/CXCL12 not only acts as a chemotactic (directed cell migration) agent, but also enhances survival of early haematopoietic cells (19). Interestingly, CD26/DPPIV, found on a number of different cell types including haematopoietic stem and progenitor cells, and present in blood plasma, can truncate SDF-1/CXCL12. We have reported that CD26/DPPIV was active in truncating SDF-1/CXCL12 into a molecule that had no chemotactic activity, and truncated SDF-1/CXCL12 blocked chemotactic response to full-length SDF-1/CXCL12 (26). Based on availability of CD26 knock-out (−/−) mice, and tri (Ile-Pro-Ile; Diprotin A)- and di (Val-Pyr)- peptide

inhibitors of DPPIV, we hypothesized then proved, that functional deletion in CD26 −/− mice, or inhibition of DPPIV *via* Diprotin A or Val-Pyr on mouse haematopoietic stem cells, enhanced the ability of the cells to better and more efficiently home to bone marrow of lethally irradiated mice. This resulted in greatly enhanced engrafting capacity, an effect specially noted when limited numbers of haematopoietic stem cells were used for transplantation (27). These results with mouse cell engraftment of lethally irradiated (28,29) and not fully myeloablated mice (30), have been confirmed and extended by others. Moreover, we (31) and others (32,33) have demonstrated similar effects for human CD34⁺ cord blood cells or G-CSF-mobilized adult peripheral blood engraftment of NOD/SCID mice. We recently demonstrated in a preliminary report (in abstract form), that DPPIV also truncated and inactivated a number of other haematopoietically active cytokines (34; unpublished data). Taken together, the results suggested that means to inhibit CD26/DPPIV activity of cells *ex vivo*, or inhibition of CD26/DPPIV in mice *in vivo*, could be considered in a human clinical trial model for testing feasibility of CD26/DPPIV inhibition to enhance engrafting capability of limited numbers of human cord blood cells present in single cord blood collections, in patients with malignant disorders. Such trials are now ongoing at the Indiana University School of Medicine under the direction of Dr Sherif Farag.

Cryopreservation of cord blood for banking

Being able to adequately cryopreserve and store/bank cord blood was and still is crucial to the clinical field of cord blood haematopoietic stem cell transplantation. Our initial laboratory study established that it was possible to cryopreserve cord blood and store it frozen with efficient recovery of the cells after thawing (2). Follow-up investigations by us have demonstrated high efficiency recovery of immature blood cells, including haematopoietic stem cells, after 5 (35), 10 (36) and 15 (37) years. Most recently, we have demonstrated that cord blood stored frozen in cryopreserved form for up to 24 years can be retrieved at high efficiency (Broxmeyer, H.E., Cooper, S., Hangoc, G. unpublished observations). While many transplantation centres may prefer not to use cord blood stored frozen for very prolonged periods of time, there is no evidence that such cord blood is any less efficient for recovery of viable stem and progenitor cells than that stored frozen for shorter periods. Our own data, which have evaluated recovery of the same exact cord blood samples at 10, 15 and up to 24 years based on pre- and post-freeze information, suggest that once cord blood is adequately cryopreserved and stored frozen, there is no significant loss of recovery of functionally active immature blood cells.

Other uses of cord blood

There are numbers of papers reporting presence and/or generation of non-haematopoietic cell types from cord blood (38) for regenerative medicine. Included in such reports are references to mesenchymal stem/stromal cells, endothelial progenitor cells and induced pluripotent stem cells. Such laboratory and pre-clinical animal studies are of interest, and can provide us with insights into biological processes. However, there is little or no rigorous evidence yet that such studies can or will translate into relevant clinical treatments. Such clinical investigation will need to be well controlled before any conclusions regarding clinical efficacy are put forth for non-haematopoietic uses of cord blood.

References

- Gluckman E, Broxmeyer HE, Auerbach AD, Friedman HS, Douglas GW, Devergie A *et al.* (1989) Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N. Engl. J. Med.* **321**, 1174–1178.
- Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D *et al.* (1989) Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc. Natl. Acad. Sci. USA* **86**, 3828–3832.
- Broxmeyer HE, Kurtzberg J, Gluckman E, Auerbach AD, Douglas G, Cooper S *et al.* (1991) Umbilical cord blood hematopoietic stem and repopulating cells in human clinical transplantation. *Blood Cells* **17**, 313–329.
- Broxmeyer HE (1997) Introduction: past, present and future of cord blood transplantation. In: Broxmeyer HE, ed. *Cellular Characteristics of Cord Blood and Cord Blood Transplantation*, pp. 1–11. Bethesda, MD, USA: Amer. Assoc. Blood Banking.
- Broxmeyer HE (2000) Introduction. Cord blood transplantation: looking back and to the future. In: Cohen SBA, Gluckman E, Rubinstein P, Madrigal JA, eds. *Cord Blood Characteristics: Role in Stem Cell Transplantation*, pp. 1–12. London, UK: M. Dunitz.
- Broxmeyer HE, Smith FO (2009) Cord blood hematopoietic cell transplantation. In: Appelbaum FR, Forman SJ, Negrin RS, Blume KG, eds. *Thomas' Hematopoietic Cell Transplantation*, 4th edn, pp. 559–576. West Sussex, UK: Wiley-Blackwell.
- Broxmeyer HE (2010) Umbilical cord blood transplantation: epilogue. In: Wagner JE, ed. *Semin. Hematol.* **47**, 97–103.
- Rocha V, Broxmeyer HE (2010) New approaches for improving engraftment after cord blood transplantation. *Biol. Blood Marrow Transplant.* **16**, S126–S132.
- Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, McGlave PB, Miller JS *et al.* (2005) Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood* **105**, 1343–1347.
- Brunstein CG, Barker JN, Weisdorf DJ, DeFor TE, Miller JS, Blazar BR *et al.* (2007) Umbilical cord blood transplantation after non-myeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. *Blood* **110**, 3064–3070.
- Ballen KK, Spitzer TR, Yeap BY, McAfee S, Dey BR, Attar E *et al.* (2007) Double unrelated reduced-intensity umbilical cord blood transplantation in adults. *Biol. Blood Marrow Transplant.* **13**, 82–89.
- Brunstein CG, Laughlin MJ (2010) Extending cord blood transplant to adults: dealing with problems and results overall. *Semin. Hematol.* **47**, 86–96.
- Broxmeyer HE, Cooper S, Hass DM, Hathaway JK, Stehman FB, Hangoc G (2009) Experimental basis of cord blood transplantation. *Bone Marrow Transplant.* **44**, 627–633.
- Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett A *et al.* (2005) Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J. Exp. Med.* **201**, 1307–1318.
- Gekas C, Dieterlen-Lievre F, Orkin SH, Mikkola HK (2005) The placenta is a niche for hematopoietic stem cells. *Dev. Cell* **8**, 365–375.
- Mikkola HK, Gekas C, Orkin SH, Dieterlen-Lievre F (2005) Placenta as a site for hematopoietic stem cell development. *Exp. Hematol.* **33**, 1048–1054.
- Rhodes KE, Gekas C, Wang Y, Lux CT, Francis CS, Chan DN *et al.* (2008) The emergence of hematopoietic stem cells is initiated in the placental vasculature in the absence of circulation. *Cell Stem Cell* **2**, 252–263.
- Robin C, Bollerot K, Mendes S, Haak E, Crisan M, Cerisoli F *et al.* (2009) Human placenta is a potent hematopoietic niche containing hematopoietic stem and progenitor cells throughout development. *Cell Stem Cell* **5**, 385–395.
- Shaheen M, Broxmeyer HE (2009) The humoral regulation of hematopoiesis. In: Hoffman R, Benz EJ Jr, Shattil SJ, Furie B, Silberstein LE, McGlave P, Heslop H, Anastasi J, eds. *Hematology: Basic Principles and Practice*, 5th edn. Part III, Chapter 24, pp. 253–275. Philadelphia, PA: Elsevier Churchill Livingstone.
- Delaney C, Heimfeld S, Brashem-Stein C, Voorhies H, Manger RL, Bernstein ID (2010) Notch-mediated expansion of human cord blood progenitor cells capable of rapid myeloid reconstitution. *Nat. Med.* **16**, 232–236.
- Campbell TB, Basu S, Hangoc G, Tao W, Broxmeyer HE (2009) Overexpression of Rheb2 enhances mouse hematopoietic progenitor cell growth while impairing stem cell repopulation. *Blood* **114**, 3392–3401.
- Han M-K, Song EK, Guo Y, Ou X, Mantel C, Broxmeyer HE (2008) SIRT1 regulates apoptosis and *Nanog* expression in mouse embryonic stem cells by controlling p53 subcellular localization. *Cell Stem Cell* **2**, 241–251.
- Mantel C, Messina-Graham S, Broxmeyer HE (2010) Upregulation of nascent mitochondrial biogenesis in mouse hematopoietic stem cells parallels upregulation of CD34 and loss of pluripotency: a potential strategy for reducing oxidative risk in stem cells. *Cell Cycle* **9**, 1–10.
- Dar A, Kollet O, Lapidot T (2006) Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. *Exp. Hematol.* **34**, 967–975.
- Spiegel A, Kalinkovich A, Shvitiel S, Kollet O, Lapidot T (2008) Stem cell regulation via dynamic interactions of the nervous and immune systems with the microenvironment. *Cell Stem Cell* **3**, 484–492.
- Christopherson KW, Hangoc G, Broxmeyer HE (2002) Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34+ progenitor cells. *J. Immunol.* **169**, 7000–7008.
- Christopherson KW, Hangoc G, Mantel CR, Broxmeyer HE (2004) Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science* **305**, 1000–1003.
- Peranteau WH, Endo M, Adibe OO, Merchant A, Zoltick PW, Flake AW (2006) CD26 inhibition enhances allogeneic donor-cell homing

- and engraftment after in utero hematopoietic-cell transplantation. *Blood* **108**, 4268–4274.
- 29 Tian C, Bagley J, Forman D, Iacomini J (2006) Inhibition of CD26 peptidase activity significantly improves engraftment of retrovirally transduced hematopoietic progenitors. *Gene Ther.* **13**, 652–658.
 - 30 Wyss BK, Donnelly AF, Zhou D, Sinn AL, Pollok KE, Goebel WS (2009) Enhanced homing and engraftment of fresh but not ex vivo cultured murine marrow cells in submyeloablated hosts following CD26 inhibition by Diprotin A. *Exp. Hematol.* **37**, 814–823.
 - 31 Campbell TB, Hangoc G, Liu Y, Pollok K, Broxmeyer HE (2007) Inhibition of CD26 in human cord blood CD34+ cells enhances their engraftment of nonobese diabetic/severe combined immunodeficiency mice. *Stem Cells Dev.* **16**, 347–354.
 - 32 Christopherson KW, Paganessi LA, Napier S, Porecha NK (2007) CD26 inhibition on CD34+ or lineage- human umbilical cord blood donor hematopoietic stem cells/hematopoietic progenitor cells improves long-term engraftment into NOD/SCID/Beta2null immunodeficient mice. *Stem Cells Dev.* **16**, 355–360.
 - 33 Kawai T, Choi U, Liu PC, Whiting-Theobald NL, Linton GF, Malech HL (2007) Diprotin A infusion into nonobese diabetic/severe combined immunodeficiency mice markedly enhances engraftment of human mobilized CD34+ peripheral blood cells. *Stem Cells Dev.* **16**, 361–370.
 - 34 Broxmeyer HE, Hoggatt J, Cooper S, Hangoc G, Pelus LM, Campbell TB (2009) CD26/Dipeptidylpeptidase IV regulates potency of selected hematopoietic growth factors through truncation, and recovery in vivo after cytotoxic stress. *Blood* **114**, 3602.
 - 35 Broxmeyer HE, Hangoc G, Cooper S, Ribeiro RC, Graves V, Yoder M *et al.* (1992) Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation of adults. *Proc. Natl. Acad. Sci. USA* **89**, 4109–4113.
 - 36 Broxmeyer HE, Cooper S (1997) High efficiency recovery of immature hematopoietic progenitor cells with extensive proliferative capacity from human cord blood cryopreserved for ten years. *Clin. Exp. Immunol.* **107**, 45–53.
 - 37 Broxmeyer HE, Srour EF, Hangoc G, Cooper S, Anderson SA, Boddine DM (2003) High-efficiency recovery of functional hematopoietic progenitor and stem cells from human cord blood cryopreserved for 15 years. *Proc. Natl. Acad. Sci. USA* **100**, 645–650.
 - 38 Broxmeyer HE (2010) Will iPS cells enhance therapeutic applicability of cord blood cells and banking? *Cell Stem Cell* **6**, 21–24.