What is the future for cord blood stem cells?

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Abstract

Stem and progenitor cells are present in cord blood at a high frequency making these cells a major target population for experimental and clinical studies. Over the past decade there has been considerable developments in cord blood research and transplantation but despite the rapid progress many problems remain. The initial hope that cord blood would be an alternative source of haemopoietic cells for transplantation has been tempered by the fact that there are insufficient cells in most cord blood collections to engraft an adult of average weight. In attempts to increase the cell number, a plethora of techniques for *ex-vivo* expansion have been developed. These techniques have also proved useful for gene therapy. As cord blood cells possess unique properties this allows them to be utilised as suitable vehicles for gene therapy and long-term engraftment of transduced cells has been achieved. Current work examining the nature of the stem cells present in this haematological source indicates that cord blood contains not only haemopoietic stem cells but also primitive non-haemopoietic cells with high proliferative and developmental potential. As attention focuses on stem cell biology and the controversies surrounding the potential use of embryonic stem cells in treatment of disease, the properties of stem cells from other sources including cord blood are being re-appraised. The purpose of this article is to review some of the current areas of work and highlight biological problems associated with the use of cord blood cells.

Ex-vivo expansion

Haemopoietic cells

Cord blood has a number of advantages as outlined in Table 1. However, the numbers of nucleated cells in the collections is limited and present a serious drawback in the routine use of cord blood cells for clinical transplantation. This has led to studies exploring ways to expand the cells *ex-vivo*. Early work was invaluable in determining suitable cell populations for initiating expansion as well as the cytokines and growth factors required for optimal amplification. It is now generally agreed that CD34+ cells, CD133+ cells or their subsets are the best starting population and possess the greater expansion potential. Several groups have reported improved conditions for *ex-vivo* expansion of myeloid progenitor cells using a range of cytokines in serum free or serum containing media (DiGiusto et al., 1996; Briddell et al., 1997; Piacibello

et al., 1998; Koller et al., 1998; McNiece et al., 2000). Expanded cells have now been used in clinical transplantation with promising results (Shpall et al., 2002). Nevertheless, some questions remain regarding cells expanded *ex-vivo*. For example, are the expanded cells functionally equivalent to their normal unmanipulated counterparts? The experimental data indicates that transplanted expanded cells can accelerate regeneration but larger numbers of expanded rather than freshly harvested progenitors are required for a similar rate of regeneration (Guenechea et al., 1999). Another issue relates to the stem cells: is it possible to maintain or expand the stem cell content *in vitro* and what is the best method of assessment? Human stem cells can engraft in irradiated mice following intravenous injection. The murine model used most widely is the NOD/SCID (non-obese diabetic/severe combined immunodeficient) which can support high levels of human cell engraftment. In this assay the

Table 1. Advantages of cord blood cells

<ul style="list-style-type: none"> • High content of primitive pluripotent cells • High content of lymphocytes with naïve phenotype • Reduction of GVHD in transplantation • Reduction in transmitting infection • Generation of progenitors and differentiated cells – myeloid and megakaryocytic lineages • Generation of cells for immunotherapy • Greater transduction efficiency • Ease of procurement

cells able to initiate and maintain the haemopoietic system *in vivo* are evaluated. The LTC-IC (long-term culture initiating cell) assay measures primitive human stem cell activity *in vitro* (reviewed in De Wynter and Ploemacher, 2001). There is at present a lack of consistency on the fate of primitive cells in *ex-vivo* culture as loss, maintenance, limited and extensive amplification of LTC-IC and NOD/SCID repopulating cells have all been demonstrated (Henschler et al., 1994; Traycoff et al., 1995; Piacibello et al., 1997; Lewis et al., 2001; Denning-Kendall et al., 2002). What is emerging is that factors allowing expansion of the most primitive cells appear to be different to those which favour expansion of the CD34+ population as a whole. Therefore, the choice of growth factors may be critical in influencing the outcome of suspension cultures (Zandstra et al., 1997). Some reports have inferred an increase in stem cell content in *ex-vivo* culture based on an increase in CD34+ cell numbers or CD34 subsets. However, relying on phenotypical analysis as an indicator of maintenance or expansion of haemopoietic stem cells may be misleading. Two recent reports cautioned that though large numbers of cells with a primitive phenotype (CD34+38-) can be generated in expansion cultures, the cells were unable to engraft or reconstitute haemopoiesis in NOD/SCID mice, emphasizing the dissociation between the phenotype of cultured cells and their function (Dorrell et al., 1999; Donaldson et al., 2001). Clearly, stable markers of haemopoietic stem cells that are maintained throughout the expansion period are desirable.

The expansion and use of progenitor cells may overcome some of the issues associated with expansion of the stem cells themselves. Apart from the myeloid cells, cord blood can be differentiated into other haemopoietic lineages that are relatively easy to grow and expand in culture (Table 2). Erythroid cells, megakaryocytes, lymphoid B-, T-, NK cells

and dendritic cells have all been generated. Dendritic cells and natural killer (NK) cells are required for immunotherapy studies. Often monocytes isolated from peripheral blood and bone marrow are used to generate dendritic cells though cord blood monocytes were found to be much less efficient in producing functional dendritic cells (Liu et al., 2001). Better results were obtained using cord blood CD34+ cells (Liu et al., 2002). This highlights the importance of assessing the initial cells to determine which is the most suitable population for generating specific lineages. *Ex-vivo* expansion of megakaryocytes would be particularly useful after cord blood transplantation where platelet recovery is often delayed. This may prevent or reduce the need for platelet transfusion support. Megakaryocyte progenitors have been expanded from CD34+ cord blood or peripheral blood progenitor cells and safely administered to patients (Bertolini et al., 1997). Logistically, for clinical transplantation cord blood cells would have to be expanded following cryopreservation. It is likely that protocols established for freshly isolated cells may need to be adjusted for cryopreserved cells.

Non-haemopoietic cells

Haemopoiesis is sustained by haemopoietic stem cells and a stromal cell system consisting of a variety of cells, including fibroblasts, adipocytes and osteoblasts. These stromal cells arise from a population of mesenchymal stem/progenitor cells (MSC) present in the marrow. The MSC have extensive self-renewal capabilities and are multipotent giving rise to bone, cartilage, muscle, stroma, fat and neural cells (Prockop et al., 1997; Pereira et al., 1998; Ferrari et al., 1998; Kopen et al., 1999). For some time, the stromal cell precursors have been identified by their ability to generate colonies of cells morphologically resembling fibroblasts in the fibroblast CFU (CFU-F) clonogenic assay (Friedenstein, 1974). This remains the standard assay for mesenchymal tissue though only a proportion of CFU-F is multipotent with multilineage differentiation capacity. Until recently cord blood was not thought to contain stromal cells, as a confluent adherent stromal layer did not develop when cord blood cells were incubated under similar conditions as those for long term bone marrow cultures (Hows et al., 1992). Moreover, no CFU-F were detectable in cord blood samples (Gutierrez-Rodriguez et al., 2000). However, a number of studies now report growth of adherent cells from cord blood which are

Table 2. Expansion and use of cord blood cells

Lineage and use		Reference
Natural killer (NK) cells	<i>Immunotherapy</i>	Condiotti et al., 2001
Erythroid cells	<i>Complimentary to blood transfusion</i>	Neildez-Nguyen et al., 2002
Dendritic cells	<i>Tumour immunotherapy</i>	Borras et al., 2000; Liu et al., 2002
Megakaryocytes	<i>Platelet transfusion</i>	Piacibello et al., 1998 Pick et al., 1998, 2002
T-cells		Robinson et al., 2002
B-cells		Ohkawara et al., 1998

able to differentiate into bone and fat cells. The adherent cells express several mesenchymal related antigens and neural antigens (Erices et al., 2000; Ha et al., 2001, Goodwin et al., 2001). The frequency of these cells is low and they do possess extensive expansion capacity similar to that of bone marrow derived MSC. They are undetectable in the standard CFU-F assay and require some modification of culture conditions for their growth. Whether the cord blood 'MSC' population is similar to the multipotent cells described in bone marrow awaits further investigation. Nevertheless, these cells could be a potential source for cell therapy.

An intriguing observation that emerged from the culture of cord blood CD34+ cells in IL-2 (interleukin-2) and conditioned medium from the 5637 bladder carcinoma cell line was that endothelial cells could be derived from CD34+ cells (Nieda et al., 1997). Subsequently, it was confirmed that endothelial progenitors exist in cord blood and can be isolated and expanded in culture (Murohara, 2001). The endothelial cells incorporate into sites of neovascularisation and form capillaries when transplanted into ischaemic rats, suggesting a possible role in cell based therapy. For some time the relationship between haemopoietic and non-haemopoietic cells was unclear but it now seems that haemopoietic and endothelial cells are related and may share a common precursor (Nishikawa, 2001).

Transplantation

More than 2000 cord blood transplants have been performed worldwide and data on transplant outcomes are beginning to accumulate (Gluckman, 2000). Unlike peripheral blood progenitor cells (PBPC), there is still no consensus on the safe dose of nucleated cord

blood cells required to provide durable engraftment even though there is a correlation between the number of nucleated cells infused and recovery. Clinical studies confirm that reliable engraftment occurs with $3-4 \times 10^5/\text{kg}$ CD34+ cells after cord blood transplantation (Wagner et al., 1996). This is approximately one tenth of the cell dose used in bone marrow transplants for successful engraftment. A recent study reduced this threshold further to $1.7 \times 10^5/\text{kg}$ CD34+ cells in recipients with HLA disparity and indicated that CD34 cell dose was of primary importance in determining the outcome after unrelated donor cord blood transplantation (Wagner et al., 2002). The same authors noted that the proportion of patients achieving neutrophil and platelet engraftment after unrelated cord blood transplant was similar to that observed after unrelated bone marrow transplant. Many investigators report a delay in platelet recovery after transplantation with cord blood that may be related to the cell dose administered (Gluckman et al., 1997; Kurtzberg et al., 1996). The importance of cell dose provides a compelling argument for focussing on ways of collecting larger cord blood units and investigating *ex-vivo* expansion of the requisite cells.

There are experimental data that transplanted expanded cells can accelerate regeneration. It has also been noted that expanded cells behave differently to freshly isolated cells resulting in delayed engraftment (Guenechea et al., 1999). In a recent clinical trial no significant delay was reported (Shpall et al., 2002). Delayed engraftment may be related to either lack of sufficient progenitors or at the cellular level it may be associated with expression of homing and adhesion molecules. Two adhesion molecules that are expressed widely on primitive cells were examined on cells amplified in suspension cultures. The results showed that although the pattern of expression of the

molecules was altered during expansion, this did not influence engraftment (Dravid and Rao, 2002). This is an important area of study as an understanding of the receptors and ligands involved in cell homing is crucial for transplantation. The molecules expressed on the cell surface could be modulated to enhance engraftment.

One of the more exciting clinical findings is that there is significantly less acute and chronic graft versus host disease (GVHD) associated with cord blood transplantation compared with identical sibling bone marrow. This finding cannot be extrapolated to unrelated donor transplants, as sufficient data on unrelated cord blood transplantation is not yet available (Hows, 2001). Little is known about the cells in the cord blood collections that mediate the GVHD effects though differences between adult and cord blood T-cells as well as their cytokine producing potential have been implicated. The fact that cord blood lymphocytes are at a different stage of maturation may also be significant. More studies are required to understand the immunoreactivity of these cells particularly if transplantation of multiple cord blood units is contemplated.

Gene therapy

The concept of gene therapy is to introduce a gene into a target cell where the gene product is then expressed leading to cure of disease. Genetic material is generally delivered into a cell using viral vectors such as retroviruses, lentiviruses and adeno-associated viruses which all integrate randomly into the genome of the recipient cells. Another viral vector, the adenovirus is maintained as an episome in the cell. Gene therapists face an awesome task. They must identify the most suitable stem cell targets, the ideal culture conditions to allow transduction and persistent expression of the transgene, with no loss of long-term engraftment potential.

Cord blood CD34+ cells have been transduced successfully with a range of viral vectors bearing a variety of markers (Schilz et al., 1998; Conneally et al., 1998; Miyoshi et al., 1999; Woods et al., 2000; Guenechea et al., 2000; Barquinero et al., 2000; Abonour et al., 2000; Salmon et al., 2000). Primitive cord blood cells are favourable as they have greater transduction efficiency in comparison to similar cells from the bone marrow. This transduction efficiency may be related to the autocrine production of cytokines by the cord

blood cells themselves which allows spontaneous exit of the cells from G₀/G₁ phase of the cell cycle (Traycoff et al., 1994). Few attempts have been made to use retroviruses for gene therapy with cord blood, possibly as the efficiency of transduction into the repopulating cells is rather low. Nevertheless, long-term engraftment of adenosine deaminase (ADA)-transduced cord blood has been achieved in a severe combined immunodeficiency (SCID) patient although to date only low levels of vector containing cells have been detected in the blood (Kohn et al., 1998). Can quiescent stem cells be induced into cell cycle and facilitate gene transduction when retroviruses are used? In a gene therapy trial on children suffering from SCID, corrective genes were packaged into retroviruses and the autologous haemopoietic cells transduced *ex-vivo*. After 11 months T-, B- and NK cell counts and function were comparable to control subjects suggesting that stem cells were transduced and the recipients were considered clinically cured (Cavazzano-Calvo et al., 2000). Unfortunately, two patients have recently developed leukaemia a reminder of the risks involved when retroviruses integrate at random into the genome. Many studies are now directed towards finding efficient non-integrating vectors capable of delivering and expressing genes in non-dividing cells.

Future prospects

The past decade has witnessed much progress in the field of cord blood research and stem cell biology. If cord blood is found to contain stem cells with pluripotent capacity this will generate new and exciting therapeutic possibilities. Consequently, these cells are being closely examined for potential treatment of a variety of human diseases. In the future a concerted effort between disciplines such as cell and molecular biology is required. Methods will have to be devised for isolation of target cells; expansion of large quantities of homogeneous stem cells; and transfer of genes into the target cells for use in the clinic. In parallel, fundamental work is required on the mechanisms influencing cell fate decisions in order to control specific lineage differentiation during *ex-vivo* expansion. Meanwhile as cord blood transplantations proceed, the level of mismatch HLA that can be tolerated without rejection will be important. Concerns surrounding the transmission of infectious agents and the likelihood of formation of tumours have already emerged and are also being addressed. The demonstration of stem cell

plasticity poses the question of what is the destiny of the infused cells after transplantation? Do they home to other tissues like some of the recent bone marrow and PBPC cell transplants? Many issues are yet to be resolved that will require rigorous assessment and robust clinical trials.

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