2 Placental Blood: New Options in Hematopoietic Stem Cell Transplantation

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2.1 Introduction

Placental tissue and neonatal cord have attracted the special attention of many different cultures throughout history, with some groups attributing deep religious importance to them. For example, in the nineteenth century, Indian tribes gave a gift of neonatal cord in turtle-shaped amulets to the newborns to ensure a long life.

It is only recently that researchers have discovered the importance of placental blood as a potential source of hematopoietic stem cells. Lethally irradiated mice could be rescued by the injection of neonatal mouse blood, demonstrating the existence of hematopoietic progenitors in cord blood (de La Sevelle et al. 1996).

E. Gluckman, in Paris, was the first to use human cord blood from a sibling donor to restore bone marrow functions in a 4 year old boy suffering from Fanconi's anemia (Gluckman et al. 1989). This revolu-

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tionary clinical result was the first to demonstrate the usefulness of placental blood in transplantation medicine. It took 3 more years of experience before cord blood could be used in a 6 year old boy with chronic myeloid leukemia to fully restore bone marrow functions after intensified regimens of high dose chemotherapy (Broxmeyer et al. 1992; Issaragrisil et al. 1992; Pahwa et al. 1994;Wagner et al. 1992). Since then cord blood has been used more than 300 times in patients suffering from inborn errors of hematopoiesis or immunological deficiencies such as Fanconi's anemia, aplastic anemia, thalassemia, sickle cell disease, X-linked lymphoproliferative syndrome and severe combined immunodeficiency disease (SCID) (Blaese and Culver 1992; Kohn et al. 1995; Kurtzberg et al. 1994; Wagner 1993). Furthermore, placental blood has been used as an alternative stem cell source for transplantation in patients with malignant diseases, e.g., acute lymphoid and myeloid leukemia, chronic myeloid leukemia and solid tumors such as neuroblastomas, after intensified regimens of chemotherapy (Broxmeyer et al. 1989; Kessinger 1992; Lu et al. 1996; Rubinstein et al. 1993). In children, cord blood has so far proven to be a realistic alternative stem cell source in addition to the clinically more established sources of autologous bone marrow or peripheral blood and allogeneic bone marrow (Broxmeyer et al. 1989; Hong and Deeg 1994; Lind 1994; Russell et al. 1993).

Although it is very early in the history of cord blood medicine, the advantages are manifold: (1) cord blood is readily accessible; (2) it is available to anyone; (3) can be obtained without any harm or pain to the mother or child after child and placental birth; and (4) contains higher numbers of hematopoietic stem cells than adult peripheral blood. Moreover the neonatal progenitor cells are readily expandable and highly proliferative in response to many growth factors and cytokines (Broxmeyer et al. 1992; Civin 1995). Thus, there are significant differences between cord blood and adult progenitor cell preparations with respect to a more advantageous source for hematopoietic stem cell separation (Mayani et al. 1993; Nimgaonkar et al. 1995).

The need for an alternative stem cell source in hematopoietic transplantation is demonstrated by the following: The use of autologous progenitor cells either from bone marrow or peripheral blood upon granulocyte or granulocyte/macrophage colony-stimulating factor (G-CSF/GM-CSF) treatment carries multiple risks such as (1) general anesthesia or growth factor treatment, (2) pain because of multiple bone

punctures to obtain bone marrow as well as (3) the risk of transplanting back into the patient contaminating tumor cells, including those from solid tumors such as mammary carcinoma or neuroblastoma (Fraig 1992; Gribben and Nadler 1993). Even purging techniques (ribozymes, cellular chromatography) do not guarantee complete tumor-cell removal from the transplant, thereby leaving the risk of early or late relapses upon autologous stem-cell transplantation. However, autologous cells are available for most tumor patients as long as marrow functions are sufficient, and with autologous stem-cell transplantation, there is no risk of immunological failure of the transplant due to graft-versus-host disease.

By contrast, the use of allogeneic cells for stem cell transplantion does not carry the potential of malignant cell contamination (Matsunaga et al. 1993). However, while allogeneic cells of matched sibling donors are the most well-tolerated in the recipient, they are available for only 30% of patients. Some 40% of patients will find a matched unrelated donor, but the remaining one third of the patients do not find a suitable donor and cannot be transplanted within a reasonable amount of time. In addition, with the transplantation of unrelated cells – even with a fully matched HLA loci – there is a high chance of acute overall graft-versus-host disease leading to lethal outcome of the transplantation and to the death of the patient (Collins and Fernandez 1994). In a large multicenter study, Kernan et al. (1993) demonstrated the limitations of using unrelated allogeneic bone marrow in 492 patients with malignant or nonmalignant hematopoietic diseases. After a median time to transplantation of 196 days, $1–5\times10^8$ mononuclear cells were transplanted in a patient cohort (median age 22 years) resulting in an engraftment probability of initially 94%. Cell recovery was achieved within 22 days after transplantation. The overall disease-free survival was 40% for low risk patients, 19% for high risk patients and 37% for patients with chronic myeloid leukemia. The rate of relapse was 19%. Most interestingly, the overall death rate was 66% in 1.5 years with 33% of these deaths caused by severe graft-versus-host diseases. Other causes of death were infections (37%), toxicity (14%), graft failure (11%) or other (15%). In summary, the transplantation of hematopoietic stem cells currently requires a source of progenitor cells that: (1) is not contaminated with tumor cells, such as occurs with autologous cell preparations, and (2) does not induce severe graft-versus-host disease (GvHD)as do unrelated stem cells, and (3) is available in sufficient numbers so as to serve

every patient. Recent clinical results indicate that neonatal blood derived from the placenta may fulfill these requirements (Rubinstein et al. 1993; Wagner et al. 1992; Wagner 1992). Clinical data from two independent studies, discussed below, support this idea.

2.2 Clinical Results

Wagner et al. (1995) presented evidence for the successful use of neonatal placental blood in the recovery of bone marrow functions in 44 children who were transplanted with their sibling-derived cord blood. Of the 44 patients 25 suffered from malignant and 19 from nonmalignant diseases. The median age was 4 years and the median weight 18.6 kg. In contrast to the much higher cell dose needed for adult stem cells, cord blood-derived stem cells became engrafted when a median of 5×10^7 (in a range of $1–33 \times 10^7$ cells) mononuclear cells per kilogram body weight were used. Patients were transplanted with fully matched grafts (n=34), with one mismatch accepted (n =4) or with two or three mismatches accepted in the HLA-A, B, C loci (n =1 and n =5, respectively). Within a median follow-up time of 1.6 years, comparable to the study presented by Kernan et al. (1993), an overall survival of 72% was observed. Causes of death were mainly infections, low frequency graft failures (n=5), GvHD (n =1) or acute respiratory distress syndrome (ARDS) (n=2). The overall prevalence of GvHD was 3% and was of low severity, responding well to corticoid treatment. Thus, this study demonstrated the feasibility of using cord blood in related patient recipients to restore bone marrow functions after high dose chemotherapy for the treatment of cancer.

A study by Kurtzberg et al. (1996) presented evidence for the clinical usefulness of cord blood in unrelated recipients. In 25 patients (n=19 malignant and n =5 nonmalignant hematologic diseases), with a median age of 7 years and a median body weight of 19.4 kg, a median cell dose of 3.6×10^7 mononuclear cells (range of $0.7–11 \times 10^7$ cells) was sufficient to provide engraftment and neutrophil recovery after a median of 22 days. Again, HLA mismatches were accepted in 24 of the 25 patients without increasing the risk of GvHD. The median time to find a donor was 115 days and the median follow-up time after transplantation was 12.5 months. Seven patients died because of infection (n =4), toxicity (n =2) or relapse (n =1). Most importantly, GvHD was not a cause of death.

In 24 of the 25 patients, no GvHD or low grade GvHD was observed in 19 patients, and five patients had grade III or IV GvHD that responded well to corticoid treatment and finally resolved. In summary, this study demonstrated a significantly lower frequency of severe grade GvHD and that GvHD did not influence the survival rate in patients transplanted with human umbilical cord blood. The relatively long time (115 days) needed to find a suitable graft can be explained by the low amount of cord blood that has been collected so far and underlines the importance of large cord blood banks all over the world to provide HLA-matched grafts for any ethnic minority and any HLA type.

A further report, by Laporte et al. (1996), described for the first time the use of cord blood in a 26 year old adult patient. Ten million mononuclear cells (MNCs) per kg resulted in the recovery of neutrophil counts above 500/ml after 23 days. Thus, bone marrow functions can be restored by numbers of MNC that are 10- to 40-fold lower than that of hematopoietic progenitor cells derived from adult bone marrow. The clinical data so far clearly demonstrate that umbilical cord blood-derived cells efficiently reconstitute bone marrow functions in patients with malignant and nonmalignant hematologic diseases. The use of cord blood-derived stem cells results in GvHD responses of low severity. GvHD in the setting of cord blood transplantation is not of life-threatening intensity but may provide enough reactivity to guarantee a graft versus leukemia (GvL) effect, which has been suggested to suppress relapse after transplantation. In the following, the characteristics of the main cell types responsible for the different results obtained with cord blood versus adult hematopoietic progenitor cell transplantation are discussed.

2.3 Hematopoietic Progenitor Cells

In vitro analyses of cord blood-derived hematopoietic progenitor cells provide evidence that CD34 antigen-expressing stem cells in cord blood contain an earlier fraction of progenitor cells that are $rh123^{low}$, $CD38^-$ and which have a high proliferative potential (Lu et al. 1993; Nimgaonkar et al. 1995; Tjonnfjord et al. 1994). When plated in methylcellulose colony assays, these cells form large colonies. Furthermore, these $CD34+/CD38^-$ cells provide long-term marrow reconstituting cells at high frequencies (Lu et al. 1993; Mayani et al. 1993; Migliaccio

et al. 1992). As a side effect of their high proliferative potential and their elevated responsiveness to hematopoietic growth factors, cord blood-derived stem cells display a comparatively high efficiency of retroviral gene transfer (Blaese and Culver 1992; Hanley et al. 1994). Our own results clearly demonstrate that, by using the magnetic cell sorting system provided by Miltenyi Biotech, Bergish Gladbach, Germany), these CD34+ cells can be separated from cord blood to a purity of 99%. In methylcellulose colony-forming assays, these cells have a threefold higher response rate in the formation of myeloid colonies when treated with interleukin (IL)-3, G-CSF and erythropoietin (EPO) compared to bone marrow derived CD34+ cells. Of the colony-forming cells, 1 in 10^4 cells displays replating capability. With respect to long-term-culture-initiating cells (LTCICs), cord blood has been demonstrated to contain 2.5-fold higher levels than adult bone marrow.

Taking into consideration that neonatal blood carries hematopoietic stem cells from either the liver or the spleen to the bone marrow of the fetus, it becomes clear that, at the time of birth, neonatal blood contains the fetal cells that are on their way to populate the neonatal bone marrow. These early and immature hematopoietic progenitor cells very easily respond to proliferative signals provided by the addition of growth factors such as IL-3, IL-1, IL-6, G-CSF, stem cell factor (SCF), FLT3, and EPO. These cytokines were tested in vitro for their effects on cell proliferation and cell expansion. A combination of IL-3, SCF and FLT3 was the most potent in expanding cell numbers in the cell culture system used. Thus, in vitro results suggest that cell expansion techniques may be beneficial in increasing cell numbers and in retaining cell quality in the transplantation of adult recipients after myeloablative chemotherapy (Broxmeyer et al. 1992). Further studies are underway to determine the characteristics and cloning potentials of the expanded cord blood cells.

2.4 Immune Cells

Another striking difference between the in vivo use of adult hematopoietic progenitor cells, either from bone marrow or peripheral blood, and cord blood is the relative lack of severe forms of GvHD in patients transplanted with the latter (Garban et al. 1996; Risdon et al. 1994). GvHD is a combination of processes that involve diverse patho-

physiological conditions (Przepiorka et al. 1995). Chemotherapy, which precedes transplantation of hematopoietic stem cells, induces tissue damage in the host and induces the release of proinflammatory cytokines, e.g., IL-1 and tumor necrosis factor-α (TNFα). Along with endotoxin, these cytokines are potent activators of host macrophages, that become activated to present antigens to surrounding T lymphocytes. These T lymphocytes, which are derived from the donor in a transplanted patient, are thus driven to further produce TH_1-like cytokines such as IL-12 or interferon (IFN)-γ, leading to acute graft-versus-host responses. Second, T lymphocytes are activated to produce TH_2-type cytokines, including IL-4 or IL-10, which primarily suppress acute GvHD but induce chronic GvHD. Cytokine dysregulation and the further activation of more potent antigen presenting cells in diverse tissues subsequently lead to targeted organ damage. Of the cytokines involved in this cascade IL-2 is important because of its ability to induce cytokine production in other immune cells and to recruit effector cells to the site of action (Przepiorka et al. 1995). Besides IL-2, it is (IFN)-γ which mediates direct cytopathic effects and which increases expression of MHC class I and II molecules on recruited cells of the immune system, thus leading to a mounted immune response. Due to this activity (IFN)-γ is thought to be the main effector of intestinal toxicity in the host (Przepiorka et al. 1995). This cytokine induces natural killer cell activity and the production of nitric oxid, further increasing cytopathic responses. By contrast, (IFN)-γ suppresses lymphocyte proliferation and thus leads to a state of immunodeficiency in the host, favoring the outbreak of opportunistic infections in the transplanted patient. Of the clinical symptoms that are associated with GvHD, IL-1 is mainly responsible for the induction of the wasting syndrome observed in the patients and TNF<a plays a significant role in the development of venoocclusive disease by inducing increased production of acute phase proteins. Thus, with regard to the reduced rate of GvHD reactions in patients transplanted with cord blood, one could hypothesize that immune cells in cord blood are less responsive to those factors that trigger GvHD. In vitro studies have demonstrated that indeed monocytes in cord blood display fewer MHC II molecules, resulting in a reduced capability to present antigen (Garban et al. 1996). Compared to adult peripheral blood-derived T lymphocytes, cord blood T cell functions are limited, i.e., helper cell activity, expression of surface molecules such as the IL-2 receptor, the production of IL-2 by activated T lymphocytes, and cyto-

toxic T lymphocyte activity (Harris et al. 1992; Roncarolo et al. 1994; Zola et al. 1995). Furthermore, cord blood-derived B lymphocytes produce low amounts of IgG or IgA as well as low numbers of MHC class II molecules (Garban et al. 1996). Thus, cord blood contains immature populations of immune cells which give rise to reduced immune responses in the presence of proinflammatory stimuli in the transplanted host.

2.5 Cord Blood Banking

Banking of cord blood may provide sufficient hematopoietic progenitor cells for allogeneic and/or autologous transplantation (Rubinstein et al. 1993). In addition to the clinical benefits of using cord blood summarized so far, a number of other benefits have to be mentioned. Cord blood is easy to obtain without any pain to the donor or risk of anesthesia or growth factor exposure. One single collection of cord blood contains sufficient cell numbers to be used in transplantation and the high proliferative quality of these stem cells indicates that even low cell numbers may be sufficient to ensure engraftment. Cord blood can be obtained in unlimited amounts and stored in liquid nitrogen without loss of cell viability for at least 8 years (Rubinstein et al. 1993). Compared to adult cells, cord blood contains a low level of infectious agents. Thus, it appears reasonable to collect and characterize cord blood for transplantation purposes. Cord blood banking comprises several steps: (1) cell collection, (2) cell processing, (3) cryopreservation, (4) analysis of cells and (5) data storage.

1. Cell collection: Cord blood can be collected within the third stage of labor with the placenta still in utero (Rubinstein et al. 1993). The alternative to cord blood collection is to obtain placental blood by gravity flow after the birth of both the child and the placenta (Rubinstein et al. 1993).
2. Cell processing: The final goal of further processing of the blood is to reduce volume and to lower storage costs. Volume reduction is sufficiently achieved by gravity sedimentation or centrifugation using hydroxyethylstarch followed by plasma extraction and separation of the white cells (Rubinstein et al. 1995). Cells are then further centrifuged and plasma is further separated (Rubinstein et al. 1995).

3. Cryopreservation: Processed cord blood cells can be cryopreserved in a final concentration of 10% DMSO, 2.5% human albumin and 5% dextran (Meagher and Herzig 1993; Rubinstein et al. 1995). Cells are initially frozen at –80°C and transferred thereafter to the liquid phase of a nitrogen tank.
4. Analyses: Cord blood cells are typed for HLA and blood group specificity. Microbiological as well as serological testing is performed for the titer of cytomegalovirus, Epstein-Barr virus, toxoplasmosis, syphilis, human T cell leukemia virus I, human immunodeficiency virus, herpes simplex virus, and hepatitis B and C viruses. Cell numbers and numbers of colony-forming cells are determined (Rubinstein et al. 1993, 1995).
5. Data storage: Data concerning blood analyses, the medical history of the parents as well as the informed consent of the parents are stored in a computerized data base according to federal safety recommendations for data storage.

In summary, based on clinical as well as in vitro data, transplantation of cord blood appears to be a new and alternative source of stem cells (Thomas 1995; Wagner 1992). Cord blood banking, once globally performed, should allow suitable progenitor cell grafts within a few days and lead to lower transplantation mortality because of significantly reduced rates of GvHD (Przepiorka et al. 1995). Thus, placental blood provides new perspectives for the transplantation of hematopoietic progenitor cells in humans.

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