

Umbilical Cord Blood, Stem Cells, and Bone Marrow Transplantation

RONALD M. KLINE

Pluripotent stem cells (PSCs) have the potential to revolutionize the future of medicine because now, for the first time, we can envision the ability to repair and replace those complex organs and tissues whose failure currently leads to the disability and premature demise of millions of people. Unfortunately, the acquisition of PSCs from early stage human embryos forces our multicultural society to attempt to reach a consensus about what constitutes human life, and at what point during embryonic development that life begins. Umbilical cord blood (UCB) is a potential alternative source of PSCs, bridging that ethical divide by providing a PSC source from a biological product that is today, with rare exceptions, simply discarded as biomedical waste.

UCB emerged in the early 1990s as a revolutionary source of hematopoietic stem cells (HSCs) that promised to dramatically improve the perennial problem of blood and marrow transplantation (BMT): the search for tissue-matched donors for patients needing an allogeneic BMT (that is, BMT from a

nonself donor). Recent discoveries in the field of stem cell research may add to the already remarkable potential of UCB by promising to make this abundant by-product of birth a source of PSCs able to treat a wide array of diseases, while continuing in its more established role as a source of the more differentiated HSCs that can reconstitute the marrow compartment of a human being.

Although UCB could provide a near limitless source of PSCs, with four million births a year in the United States alone, the advantages, disadvantages, and limitations of this PSC source compared to those derived from adult and embryonic sources remains to be fully defined in this young and rapidly progressing scientific field. In addition, the ethical concerns raised about UCB in the BMT setting also apply to its use as a source of PSCs as well, and thus are relevant to the ongoing debate about clinical applications of stem cell research.

UMBILICAL CORD BLOOD AS A SOURCE OF HEMATOPOIETIC STEM CELLS

Bone marrow transplantation was first successfully reported in 1968 in two children with immune deficiencies.¹ The successful reports followed more than a decade of failures in patients with a wide variety of diseases. In the thirty-five years since these first successful transplants, the field of bone marrow transplantation has advanced considerably, and BMT has been used to treat a variety of malignancies, hemoglobinopathies (such as thalassemia and sickle cell disease), immune deficiencies, and congenital metabolic defects. It has also emerged as a promising new therapy for a broad range of autoimmune diseases such as lupus, scleroderma, and multiple sclerosis. Nevertheless, BMT has been hindered since its first successful clinical applications in the late 1960s with the need to find “matched donors.” For a BMT to have the greatest chance of success, the major transplantation antigens of both donor and host must be fully

matched. These proteins exist in all vertebrate species and are referred to in general terms as the major histocompatibility complex (MHC). In humans they are termed human leukocyte antigens or HLAs and are genetically encoded on chromosome 6. In clinical BMT, three HLA gene products (HLA-A, HLA-B, and HLA-DRB1) have been identified as having clinical relevance. Since both a maternal and paternal allele exist for these proteins, six HLA gene products are typed in preparation for BMT and a full match is termed a “six out of six” match. Mendel’s Law of Independent Assortment dictates that the possibility that a sibling will inherit the same maternal and paternal chromosome 6 as his sibling in need of a transplant is 25 percent (50 percent chance of inheriting the same maternal chromosome 6 and 50 percent chance of inheriting the same paternal chromosome 6). Taking into account the size of the average American family, a patient needing a BMT has an approximately 30 percent chance of having an HLA match in a sibling.² Thus, relying on HLA identical siblings as the sole donor source leaves seven out of ten potential transplant patients without the possibility of curative therapy.

As a partial remedy to this problem, HLA-matched unrelated donor transplants were first attempted in 1973,³ and many successful transplants were subsequently reported.⁴ As a direct result of these efforts, the National Marrow Donor Program (NMDP) was founded in 1986 to facilitate unrelated donor transplants in the United States. Similar registries have been established worldwide. The NMDP currently has over four million U.S. donors in its data banks.⁵ When combined with the thirty-nine international registries, over six million unrelated donors are available to patients in need of a donor.⁶ Nevertheless, the NMDP is successful in identifying a donor only 75 percent of the time. The percentage is even less in minority patients, where underrepresentation of donors decreases the chances of finding a matched donor.⁷

Other alternatives to matched related and unrelated transplants include T cell–depleted bone marrow transplants and

autologous transplants (using one's own marrow as a source of HSCs). Both of these alternatives, as well as the use of unrelated donors, have significant additional risks associated with them—risks not present in matched sibling transplants—and, therefore, they have not been a universal solution to the problem of donor scarcity.

Over the last decade, a new source of HSCs has become available with the potential to significantly alleviate the shortage of donors that has plagued bone marrow transplantation since its inception. Beginning in the early 1980s, it was demonstrated that UCB contained high levels of hematopoietic progenitor cells,⁸ with a report in 1989 from Broxmeyer et al., demonstrating that the numbers of colony-forming units (an *in vitro* indicator of engraftment potential) contained in UCB collections was similar to that obtained from marrow collections where sustained hematopoietic engraftment had been achieved.⁹ This data followed two case reports from the late 1960s and early 1970s suggesting that cord blood infusions caused transient changes in RBC (red blood cell) phenotype, not related to the infusion itself, when administered in the clinical setting of conventional dose chemotherapy.¹⁰

The first umbilical cord blood transplant with sustained engraftment was performed in 1988 on a child with Fanconi's anemia, the transplant coming from his HLA identical sibling.¹¹ The child continues to do well to this day. This initial demonstration of the effectiveness of UCB in providing hematopoietic engraftment rapidly generated tremendous clinical activity centered on determining the proper uses of this virtually limitless supply of HSCs.

CLINICAL EXPERIENCE

Since the first successful cord blood transplant in 1988, the field of umbilical cord blood transplantation has expanded

dramatically with several thousand transplants worldwide since 1988. It is estimated that more than 75 percent of these transplants have used unrelated donors.¹² Initial concerns centered on the small number of mononuclear cells infused, which are generally one-tenth of the number of cells required for engraftment in more traditional forms of transplantation. These concerns have been lessened by clinical experience with umbilical cord blood transplantation, which has demonstrated successful engraftment in both pediatric and adult recipients. Nevertheless, data does show more rapid engraftment in smaller recipients of umbilical cord blood transplants.¹³

UMBILICAL CORD BLOOD AS A SOURCE OF PLURIPOTENT STEM CELLS

At this time, UCB has not been shown in either animal or human models to be a source of PSCs. It is clearly a source of HSC, and thus the potential of UCB must largely be extrapolated from data using marrow cells. These cells have been shown in preclinical models to differentiate into skeletal and cardiac muscle, hepatocytes, vascular endothelium, and neural tissue.¹⁴ Clinically, allogeneic BMT has been shown to improve osteogenesis imperfecta, a defect in the mesenchymal cells that produce Type I collagen matrix.¹⁵ It is not clear from either the preclinical or clinical models if this differentiation into nonhematopoietic tissues represents the dedifferentiation of HSCs into PSCs, or if PSCs exist alongside HSCs in the marrow compartment. If the former proves correct, then UCB will also likely be a source of PSCs. If the latter is the case, then these PSCs must also exist alongside HSCs in UCB for the potential of UCB to be realized.

At this writing, the field of stem cell research is still too undeveloped for a detailed analysis of advantages and disadvantages of one source of stem cells versus another. Significant

advances in this young field will undoubtedly clarify the picture over time and simplify what now appears to be a bewildering array of alternatives. It is also possible, as has been the case for BMT, that different stem cell choices will have different advantages and disadvantages, mandating their choice in different clinical scenarios.

HOW CORD BLOOD IS COLLECTED

Collection of UCB is a technically simple procedure that poses no foreseeable health risks to either mother or baby. The most widely used approach is to wait until the placenta is delivered and then to place the placenta in a sterile supporting structure with the umbilical cord hanging through the support. The umbilical cord is then cleansed with Betadine and alcohol, and the umbilical vein is accessed using a standard blood collection needle connected to a standard blood collection bag with anticoagulant and nutrient solution. UCB is then collected by gravity drainage, yielding approximately three ounces of blood.¹⁶ It is then cryopreserved using standard HSC techniques. In a variation on this procedure, UCB is red-cell depleted prior to cryopreservation, thus both reducing the storage volume to approximately one ounce (important when large-scale cord blood collection is envisioned) and eliminating issues of blood type and Rh factor compatibility at the time of marrow infusion.¹⁷

An alternative method involves collecting the UCB after the delivery of the child while the placenta is still in utero (the third stage of labor). Such a technique has the theoretical advantages of beginning collection earlier, before coagulation within the placenta can begin, as well as using the contractions of the uterus to enhance blood collection. These advantages are theoretical at this time, since no large comparative studies have been published. Certainly, this latter technique is more intrusive and has the potential to interfere with the mother's care after delivery.

POTENTIAL ADVANTAGES OF UMBILICAL CORD BLOOD

It appears likely that HLA matching will have just as important a role in the transplantation of PSC-derived tissues as it does for BMT. This is because the PSC-derived tissues will express human leucocyte antigens on their surface, causing them to be rejected by the host immune system if not sufficiently matched. Therefore, many of the clinical issues relevant to the use of UCB for BMT may apply to the clinical applications of PSC as well.

Size of the Potential Donor Pool. It has taken the NMDP over fifteen years to accumulate a donor pool of four million individuals. This number represents the total births in the United States in just a single year. Thus, the rapid accumulation of enough UCB samples to provide for anyone needing tissue-matched allogeneic PSC is truly an achievable goal. As an example, the New York Blood Center can provide full- or one-antigen mismatched donors for approximately half of its requests using stored blood from a pool of only 16,000 umbilical cords.¹⁸

Speed. Identifying a suitable non-cord blood, unrelated donor is a time consuming process, taking an average of four months from search initiation to marrow delivery.¹⁹ During this period, potential donors go to donor centers to have blood drawn for confirmable high resolution HLA typing and viral testing. After a donor is selected from this pool, that individual must return, pass a physical examination, and then schedule a bone marrow harvest. In contrast, cord blood has undergone viral testing upon storage, and cryopreserved DNA samples are available on-site for confirmable high-resolution HLA testing. Thus an umbilical cord blood transplant can be facilitated in as little as a few days.²⁰ For those patients for whom the acquisition of PSC-derived tissues is time critical (a patient with heart failure after a major heart attack, for instance), the rapidity with which UCB can be accessed can be lifesaving.

Racial Diversity. HLA phenotypes tend to segregate in racial groups, making it more likely that a suitable donor will come from the same racial group as the recipient. NMDP statistics show that a Caucasian will find a match 81 percent of the time, while the corresponding probabilities for African-Americans are 47 percent, Hispanics 64 percent, Pacific Islanders/Asians 55 percent, and American Indians/Alaskan Islanders 75 percent.²¹

In some cases, this represents increased HLA diversity in some ethnic groups as compared to Caucasians. For example, because African-Americans originate from the geographic area where *Homo sapiens* evolved, rather than a population subset that migrated to other continents (Europe, Asia, etc.), they have more HLA diversity in their population (and thus a more difficult time locating a suitable donor) than other ethnic groups.²² In addition, the genetic mixing that has occurred between the African-American and Caucasian populations during three centuries in North America adds still more diversity to African-American HLA phenotypes and makes it even more difficult to find suitable donors for these patients.²³

UCB harvesting can overcome these limitations both for BMT and for PSC applications by focusing collection efforts in hospitals where the children of underrepresented ethnicities are born.

POTENTIAL DISADVANTAGES

Transmission of Genetic Diseases. Cord blood harvested from the fetus represents an untested source of hematopoietic stem cells: “untested” in that the fetus has not yet demonstrated his health and viability in the external environment over several years, as is the case for other donors. Therefore, it is possible that congenital diseases, clinically unapparent in the fetus at birth, may transmit disease to recipients via the tissues derived from their PSC. These diseases could range from benign to life threatening, depending on the disease and the tissue type involved.

Many of these congenital abnormalities could be detected by the active clinical follow-up of cord-blood donors six to twelve months after birth. This would, however, require the creation of a long-term identification link between a donor and his cord blood unit, as well as continued contact with the donor center. The prospect of such a linkage has created serious privacy concerns among medical ethicists and is currently the subject of active debate.²⁴ In its place, collection centers have potential donors complete detailed questionnaires prior to UCB collection, with particular emphasis on individual and family histories of disease, as well as a detailed sexual history. If responses on the questionnaire generate medical concern, then the unit is not collected.²⁵

Clearly, the potential for transmission of genetic diseases exists for embryonic stem cells, as well as UCB, although it would not be the case for PSC derived from adult donors.

Long-term Storage. Currently, there is limited data on the viability of UCB in long-term liquid nitrogen storage. The longest that a cord-blood sample has been cryopreserved and then successfully used for BMT is eight years.²⁶ No one yet knows the limits of cord-blood viability in liquid nitrogen storage. As an approximation, it is known that cryopreserved autologous bone marrow stored for greater than two years has allowed successful engraftment in 94 to 97 percent of patients.²⁷ In one case, the marrow had been stored for eleven years.²⁸ Whether these findings can be generalized to PSC applications is unknown, and yet viability is critical to the success of all cord-blood storage efforts, both for BMT and the clinical applications of PSCs. If, for example, UCB is not viable in liquid nitrogen storage for the years it takes a person to move from infancy to being an elderly adult afflicted with the diseases for which PSC technology is currently envisioned, then clearly storage of one’s own UCB for future use would not be a meaningful option. Conversely, this would not affect the storage of UCB in donor banks where they were constantly used and replenished.

ETHICS

The availability of UCB collection efforts has raised many ethical issues apart from those of more traditional forms of BMT.²⁹ As in many other types of organ transplant, the ethical issues revolve around those of ownership, privacy, and allocation of limited resources. When applied to the clinical applications of PSCs, the issues remain largely similar.

Questions have been raised as to whether a UCB donor is entitled to reclaim his donated cord blood in the event that he or a relative needs it, and whether he is entitled to a share of the fees charged by collection banks for the UCB. This has raised UCB ownership issues, as well as the ever-present privacy concerns created by the permanent identification record that would be required. As UCB becomes a more valuable resource with the potential for stem cell generation, the above issues will only become more difficult.

In addition to unrelated UCB banks, several organizations have begun to offer, as a “for profit” service, the cryopreservation and storage of UCB. The UCB would therefore be available to that individual at a later time should he develop a condition warranting umbilical cord blood transplantation or a need for pluripotent stem cells. The largest of these companies, ViaCord, Inc., in Boston charges \$1,550 for the initial cryopreservation, with an annual fee of \$95 for continued storage.³⁰ This facet of UCB storage has raised ethical concerns about the potential availability of lifesaving technology based on economic means. To the extent that UCB proves to be a source of PSCs that have the potential to cure diseases later in life, this argument will only become stronger and more troubling.

Conversely, questions have also been raised about the ethics of marketing an expensive service to new parents when the probability of actually needing an autologous cord-blood transplant ranges upwards from one in ten thousand. Ownership issues have also been raised in the context of this service as well. Does the UCB belong to the child from whom the pla-

cental blood was taken or to the parents who presumably fund the cryopreservation and storage fees? This issue becomes relevant if the parents wish to use the UCB for a purpose other than autologous transplantation, such as for allogeneic transplantation into a sibling. In other words, do the parents of a minor child have the right to use the UCB in the best interest of a sibling (or cousin) rather than keeping the UCB cryopreserved indefinitely in case of need by the donor? Do parents have the right to sell the cord blood in a case of financial hardship? Finally, what are the rights and obligations of a storage facility if the storage fees go unpaid? Does it have the right to sell or otherwise dispose of the cord blood? Once frozen, does it have an ethical obligation to keep the cord blood in storage in perpetuity, regardless of whether or not storage fees are paid?

CONCLUSIONS

The discovery of PSCs has raised the hopes of millions of people afflicted with a wide range of diseases for which there is either no cure or a very limited one. On the other side of the argument are those who view the embryo as a human being with all the rights of an independent person. Complicating the argument still further has been the discovery of multiple sources of PSCs, with the advantages and disadvantages of each source the subject of much speculation but little data. Clearly, this knowledge will be crucial to the ongoing debate over the sources and clinical applications of PSC technology. If, for example, adult-derived stem cells prove to be as malleable as embryonic or hematopoietic stem cells, then every human will have his own fully matched reservoir of stem cells readily available, and much of the preceding discussion will be moot. If, however, embryo-derived PSCs prove to be advantageous because of their relative lack of senescence in comparison to adult-derived PSC, then cord blood–derived PSCs may

prove to be a medically and ethically acceptable alternative that will bridge the gap between the two sides of this passionate argument.

The future may bring a world in which each individual has his UCB stored at birth in anticipation of a future time when it will be used to heal a failing organ. Conversely, we may see an expansion of the mission of currently existing government-funded cord-blood banks to include such uses as the provision of HSCs for BMT and the provision of PSCs for the treatment of innumerable, previously incurable diseases. Lastly, all of the above may be irrelevant as adult-derived stem cells prove to be the equivalent of other sources, allowing each of us to repair our bodies from the stem cells residing in our own marrow compartments or adipose tissue.

NOTES

1. See R. A. Gatti et al., "Immunological Reconstitution of Sex-Linked Lymphopenic Immunological Deficiency," *Lancet* 2 (1968): 1366–69; and R. J. Albertini et al., "Bone Marrow Transplantation in a Patient with the Wiskott-Aldrich Syndrome," *Lancet* 2 (1968):1364–66.
2. See C. F. Westoff, "Fertility in the United States," *Science* 234 (1986): 554–59.
3. See B. Speck et al., "Allogeneic Bone Marrow Transplantation in a Patient with Aplastic Anemia Using a Phenotypically HL-A Identical Unrelated Donor," *Transplantation* 16 (1974): 24–28.
4. See M. R. Howard et al., "Unrelated Donor Marrow Transplantation between 1977 and 1987 at Four Centers in the United Kingdom," *Transplantation* 49 (1990): 547–53; J. L. Gawejski et al., "Bone Marrow Transplantation Using Unrelated Donors for Patients with Advanced Leukemia or Bone Marrow Failure," *Transplantation* 50 (1990): 244–49; and P. G. Beatty et al., "Marrow Transplantation from HLA-Matched Unrelated Donors for Treatment of Hematologic Malignancies," *Transplantation* 51 (1991): 443–47.
5. See NMDP website, www.marrows.org/NMDP/about_nmdp_idx.html on 6 August 2002.
6. Personal communication, Dennis Confer, Medical Director, NMDP, 1997.
7. *Ibid.*

8. See T. Nakahata and M. Ogawa, "Hematopoietic Colony-Forming Cells in Umbilical Cord Blood with Extensive Capability to Generate Mono- and Multipotential Hematopoietic Progenitors," *Journal of Clinical Investigation* 70 (1982): 1324–28; and A. G. Leary et al., "Single Cell Origin of Multilineage Colonies in Culture," *Journal of Clinical Investigation* 74 (1984): 2193–97.
9. See H. E. Broxmeyer et al., "Human Umbilical Cord Blood as a Potential Source of Transplantable Hematopoietic Stem/Progenitor Cells," *Proceedings of the National Academy of Sciences* 86 (1989): 3828–32.
10. See M. Ende, "Lymphangiosarcoma: Report of a Case," *Pac Medical Surgery* 74 (1966): 80–82; and M. Ende and N. Ende, "Hematopoietic Transplantation by Means of Fetal (Cord) Blood: A New Method," *Virginia Medical Monthly* 99 (1972): 276–80.
11. See E. Gluckman et al., "Hematopoietic Reconstitution in a Patient with Fanconi's Anemia by Means of Umbilical Cord Blood from an HLA-Identical Sibling," *New England Journal of Medicine* 321 (1989): 1174–78.
12. Personal communication, Pablo Rubinstein, Director, Placental Blood Program, New York Blood Center, 2002.
13. See P. Rubinstein et al., "Outcomes among 562 Recipients of Placental Blood Transplants from Unrelated Donors," *New England Journal of Medicine* 338 (1998):1565–77.
14. See K. A. Jackson et al., "Stem Cells: A Minireview," *Journal of Cellular Biochemistry* 85, issue S38 (2002): 1–6.
15. See E. M. Horwitz et al., "Isolated Allogeneic Bone Marrow-Derived Mesenchymal Cells Engraft and Stimulate Growth in Children with Osteogenesis Imperfecta: Implications for Cell Therapy of Bone," *Proceedings of the National Academy of Sciences* 99 (2002): 8932–37.
16. See E. Gluckman et al., "Outcome of Cord Blood Transplantation from Related and Unrelated Donors," *New England Journal of Medicine* 337 (1997): 373–81.
17. See P. Rubinstein et al., "Processing and Cryopreservation of Placental/Umbilical Cord Blood for Unrelated Bone Marrow Reconstitution," *Proceedings of the National Academy of Sciences* 92 (1995): 10119–22.
18. Personal communication, Pablo Rubinstein, 2002.
19. Personal communication, Dennis Confer, 1997.
20. Personal communication, Pablo Rubinstein, 2002.
21. Personal communication, Dennis Confer, 1997.
22. See P. H. Beatty, M. Mori, and E. Milford, "Impact of Racial Genetic Polymorphism on the Probability of Finding an HLA-Matched Donor," *Transplantation* 60 (1995): 778–83.
23. Personal communication, Dennis Confer, 1997.
24. See J. Sugarman et al., "Ethical Issues in Umbilical Cord Blood Banking," *Journal of the American Medical Association* 278 (1997): 938–43.

25. Personal communication, Pablo Rubinstein, 2002.

26. *Ibid.*

27. See H. Attarian et al., “Long-Term Cryopreservation of Bone Marrow for Autologous Storage,” *Bone Marrow Transplantation* 17 (1996): 425–30; and W. Aird et al., “Long-Term Cryopreservation of Human Stem Cells,” *Bone Marrow Transplantation* 9 (1992): 487–90.

28. See Aird et al., “Long-Term Cryopreservation of Human Stem Cells.”

29. See Sugarman et al., “Ethical Issues in Umbilical Cord Blood Banking.”

30. See ViaCord, “Letter to Participating Physicians,” 1997.