Cryopreservation of HPCs for clinical use

It used to be so "easy." Bone marrow was cryopreserved by two different methods. Areman and colleagues[1] described those techniques in their survey of current techniques for HPC processing, published in 1992. The most commonly used technique employed tissue-cultured medium supplemented with HSA or plasma and 10-percent (vol/vol) DMSO.[1,2] The bone marrow was cryopreserved by using a cooling rate of 1°C per minute, achieved with a controlled-rate freezer. The second most common method uses 5-percent DMSO with 6-percent hydroxyethyl starch suspended in balanced electrolyte solution.[3,4] The marrow was frozen in a mechanical freezer (–80°C) rather than a controlled-rate freezer.[1,3,4]

As our understanding of hematopoiesis grew, alternative sources of cells capable of reconstituting hematopoiesis were discovered and entered in clinical use. Specifically, peripheral blood progenitor cells (PBPCs) from donors whose progenitor cells had been mobilized by the use of growth factors and umbilical cord blood were commonly used to reconstitute hematopoiesis. The process of obtaining PBPCs and cord blood was different than that for bone marrow, which resulted in distinct differences in the total number of cells and the relative percentage of cell types present in the resulting component. These differences led to the studies on the processing of the cells, including cryopreservation. For example, Rubinstein and colleagues[5] described a method of RBC depletion in umbilical cord blood followed by cryopreservation in a solution containing 10-percent DMSO and frozen in a mechanical freezer (–80°C) with final storage on liquid nitrogen. Postthaw processing of the component included a dilution-and-wash step to reduce the amount of DMSO present in the infused component. This protocol differed from those previously described for bone marrow principally through the use of postthaw processing to dilute the cells and reduce the concentration of DMSO in the component to be infused. Under the auspices of the National Heart, Lung, and Blood Institute (NHLBI), a standard protocol for the processing and preservation of cord blood was developed.[6] The protocol developed included a slight variation from that developed by Rubinstein and colleagues (principally, the use of a controlled-rate freezer).

During roughly the same period, clinical studies describing adverse reactions to bone marrow of PBPC transplants were published. In an early study, Davis and colleagues[7] prospectively evaluated recipients of cryopreserved autologous transplants for toxicity. Almost all patients receiving the cryopreserved grafts exhibited dyspnea, decreased heart rates, and transient hypertension. More serious complications (oliguric renal failure and second-degree heart block) were also observed, but much less frequently. Similar results were observed in a study by Stroncek and colleagues.[8] Infusion-related reactions (principally, nausea and chills) were observed in most of the recipients of cryopreserved bone marrow. Subsequent studies documented cardiovascular complications[9,10] and neurologic events.[11] All of these studies concluded that the adverse reactions observed resulted principally from the infusion of DMSO. The larger the dose of DMSO, the higher the incidence and severity of adverse reactions.

As the number of medical indications for HPC transplantation grew, the number of facilities performing transplantation increased as well. Some of these facilities did not own controlled-rate freezers or liquid nitrogen storage tanks. Thus, economic considerations (capital and labor expenses) entered into the development of a clinical cryopreservation protocol. On the basis of results obtained by Stiff and colleagues, a variety of investigators established the ability to cryopreserve human HPCs by using a mechanical freezer (–80°C) and to achieve hematopoietic reconstitution.[12-14] It is noteworthy that these studies were performed in Europe and Asia.

In this issue of **TRANSFUSION**, Halle and colleagues[15] describe a study in which many of these issues described previously are addressed: specifically, the use of reduced levels of DMSO and monitoring of the adverse reaction associated with infusion and the use of mechanical freezers for both freezing and subsequent storage to reduce the cost and complexity of the cryopreservation process. The study's principal weakness is the lack of a control arm. It would have been very helpful to compare the outcome of the transplants under the newly developed protocol with the outcome of those under the more conventional approach, described earlier. The investigators did perform in vitro studies comparing the new protocol under investigation with the conventional protocol using controlled-rate freezing; this comparison demonstrated equivalency, but it did not extend into the clinical studies. Another strength of this article is the information on patients' tolerance of the new cryopreservation solution.

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As a cryobiologist, I was also concerned with the use of a –80°C mechanical freezer for storage of the components. For example, a cryopreservation solution containing 10-percent DMSO will not fully solidify at –80°C. Lower concentrations of DMSO will shift the value of the eutectic point for the solution to higher temperatures. If the solution is not fully solidified at the temperature of storage, the rate of cell loss increases dramatically with time in storage. Storage at higher temperatures also brings with it other changes. Specifically, biochemical activity of the cell persists until approximately –150°C. These factors can combine to accelerate the degradation of cell viability during storage to the point at which the loss limits the clinical use of the component. The financial cost of obtaining bone marrow, PBPCs and cord blood is high. In most cases, the component is irreplaceable or replaced only with great difficulty. Increasing cell losses or an increasing rate of component degradation with the use of higher storage temperatures seems hard to justify.

The only thing certain is change. The need to cryopreserve subpopulations of HPCs,[16] ex vivo expanded cell populations, and a wealth of other components that have not been developed yet is certain. In previous work, we showed that ex vivo culture changes the basic cryobiophysical characteristics of the CD34+ cells and may influence the conditions for optimal survival.[17] With change comes opportunity. New types of cell components may require or permit the development of alternative methods for cryopreserving those cells. These approaches may involve the use of novel, nontoxic, cryoprotective agents; multi-step controlled-rate freezing protocols that reduce the total freezing time from hours to minutes; simplified methods for the introduction of cryoprotective agents; or simplified, reproducible freezing of cells in a mechanical freezer. Decades of clinical experience with BMT have shown that cryopreservation protocols must first and foremost maintain a clinically acceptable level of postthaw viability. Moreover, the methods and reagents used must be appropriate for clinical applications. Finally, protocols should integrate seamlessly into clinical cell-processing protocols, and efforts should be made to make new protocols simple to implement. This will require an integrated approach, with cryobiologists working on the development of new protocols and clinical cell-processing staff working on validating and standardizing methods of cryopreservation developed in the research laboratory.

Allison Hubel, PhD
Department of Laboratory Medicine and Pathology
University of Minnesota
420 Delaware Street SE, MMC 609
Minneapolis, MN 55455

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