Peripheral blood stem cells—collection and storage

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Increasingly, peripheral blood is being used in place of bone marrow as an autologous source of hematopoietic stem and progenitor cells. In part, this reflects the continued documentation that the posttransplant recovery of neutrophils and platelets is more rapid with peripheral blood stem cells (PBSCs) than with bone marrow (by 1–2 weeks). PBSC collections are performed after mobilization of stem cells into the circulation by chemotherapy and/or growth factors. The growth factor, granulocyte colony stimulating factor (G-CSF), has been shown to have the most marked effect, increasing the progenitor cell level by as much as 100-fold. Granulocyte-macrophage-colony-stimulating factor (GM-CSF) is also being administered to mobilize stem and progenitor cells into the circulation.

The potential for allogeneic transplantation is receiving increasing attention worldwide. For this mode of therapy, stem cells are collected from healthy donors by apheresis after G-CSF mobilization of stem cells from the bone marrow. G-CSF is being injected subcutaneously for 4 to 5 days at doses of between 5 and 16 μg/kg body weight/day. It was reported at the meeting of the International Society of Experimental Hematology in August 1996 that over 500 allogeneic transplantations had been performed in Europe by the end of 1995. In the United States, the number of procedures is probably over 200. The National Bone Marrow Program (NMDP) is coordinating a study of the use of PBSCs from unrelated donors as a second transplant when a primary bone marrow transplant does not result in engraftment. PBSCs donated by HLA-matched siblings and unrelated donors have been used for some initial transplants and, no doubt, will receive increased consideration in the future. The treatment of healthy donors with G-CSF is associated with a variety of adverse effects, notably bone pain, as a result of the growth factor influencing the bone marrow matrix. The adverse effects disappear shortly after the G-CSF treatment is completed. There is concern about the potential long-term effects of G-CSF administration because of the possibility (as yet unobserved in healthy donors) that the growth factor could increase the potential for leukemia or another type of malignancy.

Principles of Collection

Currently, collections of PBSCs are being performed predominantly using the Spectra (COBE BCT, Lakewood, CO) and CS-3000 (Fenwal Laboratories, Deerfield, IL) instruments. Specific programs provide for the collection of mononuclear leukocyte preparations that contain stem cells. Dual needle procedures are being used with both instruments. Venous access is preferentially performed using antecubital veins. However, for many autologous collections, it becomes necessary to utilize central lines inserted into the subclavian, jugular, or femoral veins. Some healthy donors may also require central lines.

Collection Procedures

There is substantial variation in the practices used to collect PBSCs for autologous use. In part, this is necessary because characteristics relating to the rate and extent of mobilization of stem cells in patients can vary dramatically. On the other hand, because PBSC transplantation is still an emerging therapy, optimal practices have not been identified.

Following an appropriate mobilization regimen, 2–5 apheresis procedures, each involving the processing of approximately 10–15 liters of whole blood, are needed to collect a cell dose prescribed by the treating physician. In part, the number of procedures can depend on the objectives set forth for a specific patient by the treating physician. Currently, the goals for autologous collection are 5–8 x 10⁶ mononuclear cells/kg body weight and/or 2–6 x 10⁶ CD34+ cells/kg body weight. To reduce the number of apheresis collections needed, there is increased use of large-volume leukapheresis procedures in which the volume of blood processed at one time is greater than 15 liters; as many as 30–40 liters of whole
blood are being processed at one time. One benefit is that increased blood processing, in itself, has a CD34+ cell-mobilizing influence. Even with this approach, there is a marked variation in the number of cells that are collected from patients. In a recent study, 28.9 ± 4.9 L of blood were processed from 14 previously treated cancer patients. The median mononuclear leukocyte count/kg body weight was 2.5 \times 10^9 (range 1.0–7.4) and the median CD34+ count was 2.8 \times 10^6/kg body weight (range 0–112.5). A median of two procedures (range 1–4) was needed to collect the target mononuclear cell level from 13 patients, with 8 of the 13 requiring only one or two procedures.4

The procedures for autologous collection of PBSCs were developed for adult patients.5 However, it has been determined that G-CSF effectively mobilizes PBSCs in children with malignancies. A recent study showed that 1 or 2 apheresis procedures provided the minimum of 5–7 \times 10^6/kg body weight with pediatric patients ranging in age from 2–15 years and in body weight from 12 to 60 kg. The volume of blood processed ranged from 117–540 mL/kg.5

The cellular characteristics of PBSC components collected from patients with the CS-3000 and Spectra instruments appear to be comparable. Some differences in mononuclear cell yield, efficiency of mononuclear cell collection, residual red cells, and granulocytes have been reported. A recent report compared the properties of CS-3000 and Spectra components in a well-controlled study.7 The patients were homogeneous with respect to disease and treatment therapy. Apheresis whole blood flow rates and scheduled time per procedure were comparable. Mononuclear cell levels and colony-forming unit assay results were statistically similar. Statistically significant differences in hematocrit and the number of granulocytes and platelets were observed. However, it was concluded that the differences were not "physiologically important."7

The volume of collected CS-3000 and Spectra components are different because of instrument differences. When approximately 10–15 liters of whole blood are processed, the volume of a CS-3000 component will be approximately 55–60 mL and the volume of Spectra components will be in the 110–200 mL range. A variety of post-collection practices are in use to modify the volume of components. In some instances, components are diluted with autologous plasma while in other cases a concentration step is used.

**Storage Topics**

*Storage under liquid conditions (non-freezing)*

When PBSCs are to be stored for any extended length of time, they are cryopreserved. However, short-term liquid storage is possible. Some facilities add tissue culture fluid or a solution approved for in vivo infusions such as Plasmalyte A (Fenwal Laboratories, Deerfield, IL). Documentation that this practice has merit has not been published. Recent publications have indicated that levels of the progenitor colony, CFU-GM, are well maintained for 1–2 days at 1–6˚C. One report indicates that rapid engraftment occurs after autologous transplantation following a 4-day storage period at 4˚C.8 The NMDP protocol for donation of PBSCs for second transplants stipulates that the preparations should be stored at 2–6˚C and transfused within 48 hours of collection.
Cryopreservation

PBSCs are being cryopreserved using methods that were initially developed for bone marrow. Increasingly, PBSCs are being frozen and stored in plastic blood containers and not in vials. The most widely used procedure employs the cryoprotective substance, DMSO, at a final concentration of 10%. The PBSCs are slowly diluted (1:1) with a 20% DMSO solution, cooled to 4°C, and then frozen using a controlled-rate freezer initially at -1°C/minute. They are stored in the liquid or vapor phase of liquid nitrogen. The nucleated cell concentration in the suspension to be frozen has not been optimized. It has been presumed that too concentrated a suspension could lead to cell clumping. Many laboratories dilute to $2 \times 10^8$ cells/mL, usually with autologous plasma. A recent study showed retention of progenitor colony viability and maintenance of engraftment potential over a wide concentration range of approximately 1–8 $\times 10^8$ nucleated cells/mL.9

Mechanical freezing of PBSCs is also being utilized. This alternative to controlled rate freezing uses a cryoprotecting solution consisting of hydroxyethyl starch, DMSO, and albumin (final concentrations in cell suspensions of 6%, 5%, and 4% respectively).10,11 A 1-to-1 mixture of cell suspension and cryoprotecting solution is placed directly into a mechanical freezer at -80°C or occasionally at -135°C, for freezing and subsequent storage. The bags of cell suspension are placed in aluminum frames, such as those used for red cell freezing. The canisters are covered with styrofoam to maintain the freezing rate at approximately 1–3°C per minute.

In summary, PBSCs collected by apheresis have become the main source of hematopoietic cells for autologous transplantation. Clinical studies have shown that autologous PBSC transplantation leads to a more rapid engraftment, assessed by platelet and neutrophil recovery, than bone marrow. In addition, it is well accepted that use of PBSCs is associated with sustained long-term production of the various cell lineages. Autologous PBSCs can be stored for short periods of time under liquid conditions prior to cryopreservation or transplantation. PBSCs can be frozen using controlled rate procedures or mechanical freezers. The use of allogeneic PBSCs is still experimental. The need to administer G-CSF to healthy donors to mobilize stem cells from the marrow has raised some concerns, especially relating to the lack of data on long-term effects. It is expected that allogeneic PBSC transplantation will be used increasingly in coming years.

References