The Proper Use of Previously Frozen Red Blood Cells for Transfusion

By Hugh Chaplin, Jr.

The SINGLE incontrovertible indication for transfusion of frozen–stored red blood cells (FS-RBC) is: to provide readily available rare donor blood for patients allo-sensitized to high-frequency donor antigens. Other alleged indications have not been adequately documented. It is useful to examine the background for so restrictive a definition.

In 1950, it was first demonstrated that human red blood cells (RBC) could be stored frozen for prolonged periods, thawed, washed free of cryopreservative, and transfused with normal in vivo survival of 85%-90% of the recovered cells. In the first blush of excitement at achieving the science-fiction goal of suspended animation, it seemed possible that frozen storage of RBC might be the panacea for most of the problems that beset the transfusion field. In particular, it should be possible to stockpile blood in large quantities and for prolonged periods, eliminating the problem of seasonal shortages and providing an important resource to meet unexpected major local or national catastrophes; another blessing would be the establishment of enduring supplies of rare donor bloods so that they could be made available in sufficient numbers whenever needed.

During the succeeding 20–25 yr, RBC freezing technology was refined, −80° air refrigerators and −140° gas phase liquid nitrogen storage containers were mass produced, mechanical cell washers for cryopreservative removal were developed, FDA standards were issued, and the number of units of FS-RBC escalated to more than 100,000 annually. It appeared that the 1950 dream was coming true! During this developmental period, increasing numbers of virtues were ascribed to FS-RBC when compared to conventional whole blood and packed RBC: (1) they could be transfused uneventfully to patients experiencing frequently recurring febrile transfusion reactions secondary to sensitization to donor leukocytes and platelets; (2) they were well tolerated by the rare patients who experienced serious anaphylactic reactions to donor IgA globulin; (3) they produced a lower incidence of sensitization to buffy-coat elements in patients requiring repeated transfusion over prolonged intervals, a particularly desirable feature for prospective transplantation recipients; (4) in one prospective study they appeared to have a reduced likelihood of transmitting serum hepatitis; (5) the ATP and 2, 3-DPG content of FS-RBC were close to the content in fresh donor RBC and therefore made them a "superior" oxygen-carrying product compared to ATP- and 2, 3-DPG-depleted RBC in donor units that had been stored in blood bank refrigerators at 4°C from 7 to 21 days. With credentials like these, could the day be far off when frozen storage would be the preferred disposition of most of the 8–10 million units donated annually by the nation's volunteers?

Four major deterrents persistently dampened the wave of enthusiasm for expanded frozen RBC storage. First, FS-RBC were cumbersome to prepare. The freshly drawn unit had to be centrifuged and its plasma removed and substituted by a cryoprotective preservative solution; the contents were then transferred to a separate larger plastic container capable of withstanding temperatures of −80°C and below, and the unit was frozen and stored in a bulky low-temperature freezer. When needed, the frozen unit was thawed in a 37°C waterbath and the contents transferred to a "washing machine" where the cryopreservative (generally 40% glycerol) was removed by a variety of dilutional washing procedures employing multiliter volumes of progressively less hypertonic buffered crystallloid and sugar solutions. Finally, the product was transferred to another plastic container, suitably labeled and ready for transfusion. When contemplated on a large scale, the procedures would clearly demand major increases in the time, space, and manpower required to furnish the very large numbers of donor units required in operating rooms, emergency rooms, and at the bedsides of the population to be served.

A second deterrent was the high cost generated by the complex preparative procedures just described: technicians' salaries, plastic softwares, cryoprotective and washing solutions, refrigerants, special refrigerators and mechanical washing devices, plus the increased space required for all of the above. Estimates place the cost of one unit of FS-RBC at 2–4 times the cost of a conventional unit of whole blood or packed RBC.

A third deterrent was the short shelf-life of FS-
RBC, which must be transfused within 24 hr of processing because of the potential for bacterial contamination incident to the numerous transfers required during the multistage preparative procedures. A fourth deterrent was the loss of one-fifth to one-fourth of the RBC secondary to freeze–thaw lysis, mechanical losses in the numerous transfers and in the washing procedure, plus irreversible damage to approximately 10% of the recovered cells resulting in their removal from the recipients’ circulation within 24 hr of transfusion. This 75% “index of therapeutic effectiveness” (100% minus the sum of in vitro and in vivo losses) was disappointingly low for so costly a product.

Despite the restraints imposed by the deterrents just enumerated, there was a steady increase in the number of FS-RBC processed by the American Red Cross Blood Services system over the years, peaking at 95,214 in 1978/79. A significant but smaller number of units were being frozen–stored in community and hospital blood banks. In the succeeding 3 yr, the number of Red Cross FS-RBC has decreased by almost 50%. The principal reason for the current decline in FS-RBC usage lies in the changing views about the role of transfusions vis à vis long-term survival of transplanted cadaver grafts. The conventional wisdom that sensitization to donor histocompatibility antigens (primarily on blood donor WBCs and platelets) could prejudice renal allograft survival had led to increasing reliance on FS-RBC to meet the transfusion needs of potential graft recipients. The rationale for this practice was that FS-RBC contained the smallest number of WBCs and platelets of any of the buffy-coat poor RBC products currently available and, therefore, would presumably be least likely to provoke the presumed prejudicial immune response. In 1978, Opelz and Terasaki reported that in a study of 1360 cadaver donor transplants, 4-yr graft survival in never-transfused recipients was 30% ± 3% in contrast to 65% ± 5% in recipients who had received > 20 pretransplant whole blood or packed RBC transfusions. Generally confirmatory findings were reported from other centers in the United States and abroad, suggesting that prior exposure to donor histocompatibility antigens actually enhanced, rather than prejudiced, donor graft survival. Consequently, the transfusion of FS-RBC to potential renal graft recipients has begun to decline, although some dialysis centers continue to rely largely upon FS-RBC, primarily in the hope that this will minimize transfusion-transmitted hepatitis in the dialysis populations.

Because FS-RBC are expensive and cumbersome to prepare, the alleged virtues of FS-RBC in comparison to less costly and more easily prepared RBC products must be carefully reexamined. With the exception of near normal ATP and 2, 3-DPG levels, almost all the “superior” qualities attributed to FS-RBC are not a consequence of their low-temperature storage, but rather reflect the extensive washing required to remove the cryoprotective agent. The notion that FS-RBC carry a reduced risk of transmitting hepatitis is an important example, since it is the persistence of this notion that is responsible for most of the utilization of FS-RBC in the United States today (largely in dialysis centers). Support for a reduced hepatitis risk derives from a single relatively small prospective study reported in 1970 that utilized a wash protocol that is uncommonly used now. A retrospective review of 3000 FS-RBC transfusions in a dialysis unit supported a reduced risk of hepatitis compared to what would be expected for whole blood or packed RBC, but conditions of the study made it difficult to draw definitive conclusions. It is known that the incidence and severity of serum-transmitted hepatitis is dose-dependent. Therefore, the dilutional effect of the washing procedure should result in reduced risk of hepatitis transmission. Since it is clear that the freeze–thaw and storage conditions do not inactivate hepatitis virus, it is logical to predict that equivalent washing of fresh, never-frozen donor blood should provide equivalent reduced hepatitis risk, at reduced expense, and without the freeze–thaw RBC losses and reduced 24-hr in vivo survival that contribute to the relatively low “index of therapeutic effectiveness” of FS-RBC. Prospective studies to test this prediction are urgently needed.

Similar considerations obtain for the claim of reduced sensitization to histocompatibility antigens in recipients of FS-RBC. Here again, one is dealing with a dose-dependent phenomenon. Mechanical RBC washing devices are now able to reduce the number of residual donor leukocytes in never-frozen RBC close to the low levels observed in FS-RBC. While the absolute number of clearly identifiable WBC in FS-RBC continues to be somewhat lower than in washed never-frozen RBC (<5% versus 10% of original donor WBC), FS-RBC contain fragments of disrupted WBC and platelets, the amount and immunogenicity of which have not been well defined. Thus, definitive prospective comparisons of histocompatibility antigen immunogenicity of FS-RBC versus washed never-frozen RBC must be carried out to support any claim for the superiority of FS-RBC in this regard. With respect to providing a RBC product well tolerated by recipients experiencing buffy-coat-related febrile transfusion reactions, washed never-frozen RBC containing <10% of original donor WBC are as well tolerated as FS-RBC and have the advantage of lower cost and higher “index of therapeutic effectiveness.”
Defining the clinical circumstances requiring near-normal levels of RBC ATP and 2, 3-DPG for transfusion falls outside the scope of this review. Available evidence suggests that such circumstances represent a small proportion of total transfusion usage.  

Because of its lower cost and higher "index of therapeutic effectiveness," fresh donor blood or blood stored up to 7-10 days in the currently improved CPD-A preservative solution is preferable to FS-RBC in these special circumstances.

To return, then, to the single incontrovertible contribution of FS-RBC—namely, provision of rare donor blood for transfusion to recipients previously sensitized to high-frequency donor RBC alloantigens—a number of points should be emphasized. Fortunately, the number of patients falling into this category is very small, representing a fraction of 1% of the nation's annual RBC utilization. To meet the requirements for rare donor bloods, a small number of frozen storage depots well scattered geographically will suffice. If the prospective recipient is near enough to a center, the unit can be processed free of cryopreservative at the frozen storage depot and transported (at 4-10°C) to the patient within the 24-hr shelf-life of the product. If the patient lives too far from the frozen storage depot, the unit may be transported in the frozen state and processed free of cryopreservative at a facility close to the recipient. Methods have been described for cryopreservative removal by batch-washing, requiring nothing more elaborate than a standard blood bank centrifuge capable of spinning down a conventional unit of donor blood.

An even better utilization of RBC freezing technology in meeting the rare donor needs of allosensitized recipients is its use to establish a “bank account” of the patient's own blood during a period when the patient's hemoglobin is normal; such blood can be utilized for autotransfusion should a subsequent need arise. This procedure may also be used for selected patients with autoimmune hemolytic anemia in remission, so that safe RBC are available for autotransfusion during subsequent relapses, when reliable compatibility testing may be “impossible” because of strong autoantibody in the patient's serum.

To summarize, the progressively refined technology for frozen RBC storage has been an illustrious achievement over the past 35 yr. Ironically, its most important impact may prove to be its contribution to evolving conditions for frozen preservation of bone marrow precursor cells, tumor cell lines, hybridoma cell lines, platelets, and selected leukocyte populations. The extent of its role in meeting the need for transfused RBC has not yet been resolved. It is clearly of great value in meeting the needs for rare donor RBC. Its practical value and cost-effectiveness in a variety of other circumstances must await well designed studies to demonstrate whether or not FS-RBC are superior to RBC depleted of plasma, white cells, and platelets by less costly and less wasteful methods than are involved in the total freeze-thaw-washing process.

REFERENCES

The proper use of previously frozen red blood cells for transfusion

H Jr Chaplin