Hematopoietic Supportive Care

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Introduction

Over the past 35 or more years, mature and immature allogeneic and autologous hematopoietic cells collected from the PB have been used in the supportive care management of patients with therapy or disease-induced intermittent, recurrent, and often prolonged periods of BM suppression. Clinical, laboratory, and technological research has been directed into the cellular contents of the intravascular and extravascular space, or laboratory, and technological research has been directed into the cellular contents of the intravascular and extravascular space, where cell populations of interest in quantities ranging from \(10^6-10^{12}\) were available for use. It is most fitting today that we pause to recognize and honor Dr. Emil Freireich for his major role in defining the principles of hematopoietic supportive care in the 1960s and 1970s, explore the pursuit and expansion of those principles by his students and colleagues in the 1980s and 1990s, evaluate how the principles derived from those studies may remain relevant in today's management of myelosuppressed patients, and speculate on the future of supportive care.

Supportive care concepts began with the investigation of the treatment and biology of AL in the 1950s. As so aptly stated by Dr. Freireich, "When we started working on leukemia, a disease whose prime manifestation is BM failure, we realized that the morbidity and mortality from the disease was accounted for almost entirely by hemorrhage and infection" (1). He recognized that replacement of physiologically appropriate quantities of granulocytes and platelets required research into the biology, kinetics, and vascular distribution of these cells once they were transfused into neutropenic and thrombocytopenic patients, and his realization that single units of whole blood contained inadequate quantities of cells for transfusion led to his direct participation in the development of CF-BCS centrifugal technology, by which large volumes of donor blood could be processed for specific cell collection (2).

Among the transfusion principles first described by Freireich for leukocytes but subsequently shown to be applicable to all other PB cell populations used for hematopoietic support were that the quantity of cells recovered in the patient's PB related to the dose of cells transfused, and the circulating PB level of the cell was crucially related to the biological effects (3). Clinical and immunological factors that might alter the observed response from the expected response would be demonstrated subsequently.

Granulocyte Concentrate Transfusions for Neutropenic Patients

History. Early observations by Hersh, Bodey, and Freireich (3, 4) that risks of infection increased when the PB PMNs dropped below \(5 \times 10^9/\text{liter}\) and that neutropenic patients responded to transfusions of leukemic granulocytes (PMNs) collected from CML patients led, in the early 1970s, to therapeutic replacement trials for established infection in neutropenic AL patients unresponsive to antibiotics with PMNs collected from related NDs. On the basis of these studies, it was estimated that quantities of \(10^{11}\) PMNs might be needed to provide circulating PMN levels \(\geq 5 \times 10^9/\text{liter}\), but first-generation CF-BCS provided average leukocyte quantities of \(\leq 20 \times 10^9/\text{cells}\) from NDs. With observed recoveries of 5-15% of the transfused cells, these doses would prove inadequate to raise PB PMNs to desired levels. Relationships between the donor leukocyte count, the volume of blood processed, and efficiency of CF-BCS technology and leukocyte yields had been established, but two events occurred in the 1970s to improve PMN yields.

Studies by McCredie (5) and others demonstrated that inducing granulocytosis in donors with steroids or etiocholanolone (a steroid metabolite) and adding a high molecular weight erythrocyte sedimenting agent, HES (a highly branched polymer of glucose and a chemical analogue of glycogen), to donor blood during CF-BCS processing resulted in significantly improved PMN yields.

The relationship between donor/patient ABO and HLA compatibility and transfusion response was also demonstrated in the 1970s. Granulocyte increments in the immediate posttransfusion period were highest in ABO and HLA (Loci A and B) compatible donor/recipient pairs (median = 813 PMNs/\(\mu l\)), and lowest in ABO and HLA-incompatible pairs (median = 290 PMNs/\(\mu l\); Ref. 6). Because of the short half-life of PMN, daily transfusions of PMN were necessary, and patients received 1 to 20 or more transfusions, depending on donor availability and patient status. The failure to sustain excellent recovery for some patients over a series of transfusions was postulated to be related primarily to the onset of alloimmunization from exposure to multiple donor leukocyte antigens in the PMN concentrates as well as the multiple platelet and erythrocyte transfusions also required by the patients.

Therapeutic PMN replacement could be shown to be beneficial to neutropenic patients with Gram-negative bacterial sepsis in the presence of continuing marrow aplasia (7), but the inability to clearly predict the population at risk who would benefit from PMN replacement, the lack of adequate numbers of compatible donors, the failure to demonstrate statistically significant relationships between PMN transfusion and patient ben-


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3 The abbreviations used are: PB, peripheral blood; PBSC, PB stem cell; AL, acute leukemia; CF, continuous flow; BCS, blood cell separation; CML, chronic myeloid leukemia; ND, normal donor; HES, hydroxyethyl starch; HLA, human lymphocyte antigen; G-CSF, granulocyte colony-stimulating factor; SDPC, single donor platelet concentrate; BV, blood volume; MNC, mononuclear cell; PC, platelet concentrate; BM, bone marrow; DLI, donor lymphocyte.
efit, and improvements in antibiotic management led to a decline in the use of PMN transfusions in the 1980s.

**Present and Future.** Several factors contributed to a renewed interest in the use of PMN concentrates in the 1990s: (a) infection remains a major contributing factor to morbidity and mortality, with fungal infections predominating over bacterial infections (8); (b) research with hematopoietic growth factors, such as G-CSF, has demonstrated an increased killing effect of G-CSF and PMN toward hyphae of *Candida* and *Aspergillus* organisms in neutropenic animal models and in *in vitro* experiments (9); (c) the use of G-CSF, which provided a brisk leukocytosis and shortened periods of neutropenia in patients, formed the basis for investigating its use in NDs to augment PMN yields (10); and (d) the formulation of low a molecular weight HES to collect PMNs offered pharmacological advantages to donors over high molecular weight HES.

Current clinical trials of G-CSF-primed donor PMN transfusions face the same problems encountered the 1970s. Patients have advanced infection, usually pneumonia, sinusitis, or disseminated fungal infection, prolonged neutropenia, and have failed to respond to current antibiotic and cytokine management. Results of 124 PMN transfusions from 35 G-CSF-primed related donors to 15 neutropenic patients with established fungal infections have been reported by Hester et al. (11). With median doses of 55 ± 23 × 10⁹ cells/transfusion and recoveries of 5–15% of the cells posttransfusion, PB PMN levels averaged 1.0 × 10⁹/liter, a significantly higher increment than that of earlier studies, and a level estimated to be physiologically appropriate for controlling infections. Complete resolution of these established fungal infections was not observed or expected, although partial responses were demonstrated in 11 patients. Responses were primarily observed in patients who had shorter periods of neutropenia and infection (median = 15 and 8 days, respectively) rather than in the nonresponders (median = 25 and 17 days, respectively). Given these encouraging results, new protocols for prophylactic replacement to AL patients identified to be at high risk of fatal infections are underway.

**PC Transfusions for Hemostasis**

**History.** The basic objective of prophylactic platelet replacement to thrombocytopenic myelosuppressed patients is to provide hemostasis in an effort to reduce risks for major and/or fatal hemorrhage: the risks of fatality are greater for patients with central nervous system or major pulmonary hemorrhage than for those with gastrointestinal, genitourinary, or mucocutaneous hemorrhage. The level of thrombocytopenia generally accepted to correlate hemorrhagic risks was defined in the 1960s to be a PB platelet concentration of ≤20 × 10⁹/liter (12). A consensus was not reached, however, as to the PB platelet level required to achieve hemostasis, although Djerassi and Farber (13) reported in 1965 that hemostasis was most consistently apparent in patients whose posttransfusion platelet count was increased by 40 × 10⁹/liter.

The fundamental task would seem to be to define the total dose of platelets required to achieve this PB concentration. Studies of fresh SDPCs collected by CF-BCS technology in the early 1980s and transfused to AL patients demonstrated that for SDPC doses averaging 1.0 × 10¹¹ platelets/liter patient BV, an average of 40% of the transfused platelets could be recovered in patient’s PB. This provided a platelet level averaging 40 × 10⁹/liter that correlated with a corrected template bleeding time (14). Recovery was influenced by clinical and immunological factors, however, and ranged from almost 100% recovery in asplenic patients, to 50–80% in afebrile nonimmunized patients, to 20–40% in patients with splenomegaly or patients with hyperpyrexia, and to 0–20% in alloimmunized patients. For the nonalloimmunized patient with posttransfusion platelet counts of ≤40 × 10⁹/liter, an increase in transfusion dose of >1.0 × 10¹⁰/liter BV would suffice to provide adequate increments. For the alloimmunized patient, compatible donors must be identified. The relevance of the HLA system to alloimmunization was recognized by Yankee (15) in the 1970s, although selected HLA-matched donors are now reserved for alloimmunized patients, because numerous studies demonstrated that HLA compatibility was not essential for nonimmunized patients. Immunological factors responsible for alloimmunization remain unresolved and are linked to issues of selection of SDPCs versus random PCs for transfusion, the inability to predict which patients are at risk for alloimmunization, the number of transfusions required to become immunized, the time interval to immunization, and which antigens are most relevant to immunization. Evidence accumulated, however, that leucocyte contamination in PCs, primarily MNCs, which ranged from 10⁷–10⁹ cells/PC collected in the 1970s and 1980s, were likely responsible for most alloimmunization and also served as a source of transmission of infectious particles to patients.

**Present and Future.** BCS technology in the 1990s has reduced the MNC contamination to levels of 10⁴–10⁵ cells/PC, and studies are already emerging that show significant reductions in refractoriness to transfusion and formation of lymphocytotoxic antibodies as well as a reduction in transmission of cytomegalovirus to neonates and BM transplant patients (16, 17).

To overcome residual problems of alloimmunization and ethnic diversity in patient populations for whom no compatible donor can be identified, new approaches will be required. Studies of autologous and allogeneic compatible donor cryopreserved PCs for support of patients through maintenance, reinduction chemotherapy, and/or transplant strategies have been reported, although this approach remains underused (18). The introduction of thrombopoietin to stimulate megakaryopoiesis will undoubtedly diminish platelet transfusion requirements for some but not all of the patients. Infusions of lyophilized infusible platelet membrane vesicle fragments prepared from human platelets may prove to be an alternative source of hemostatic material (19). Anderlini et al. (8) also reported that hemorrhage remains a contributing factor to the death of some AML patients undergoing induction chemotherapy, suggesting that careful analyses of patient clinical data integrated with information of transfusion support will be crucial for future studies. Difficulties may be encountered in pursuing research in transfusion hemostasis, because current guidelines for platelet production consider blood as a manufactured product, not a biological therapy.

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4 Unpublished data.
PBSC Transplant

History. That some limited quantities of PBSCs circulated in the PB had been known for many years, but it was not clear that they were capable of trilineage hematopoietic reconstruction. Exclusion of patients with refractory multiple myeloma from intensive chemotherapy/autologous transplant regimens because of inadequate BM harvests or marrow infiltration with malignant cells formed the basis for initiation of investigations of the role for PBSCs in transplant strategies in the mid-1980s. Patients in these early studies had received extensive prior chemotherapy, and they were primed only with low-dose Cytoxan (1.0 g/m²). Leukapheresis was initiated during rebound leukocytosis. Theorizing that PBSCs lay within the small PB lymphocyte, a target dose of 4 × 10⁹ lymphocytes/kg was set for collection, requiring an average of 10 or more leukaphereses per patient. All patients engrafted (20).

Several concurrent and interrelated factors emerged to shift transplant protocols from BM to PBSCs. These included: (a) identification of the CD34+ antigen as a marker of the PBSC population, which allowed a more rapid assessment of stem cell quantities by immunofluorescent methods than did the conventional 2-week colony-forming unit cultures; (b) correlations between the CD34+ dose/kg and patient myeloid engraftment data that permitted the relationships between the two variables to be quantitated so that the number of leukapheresis procedures required could be based on a targeted dose of CD34+ cells; (c) the advent of cytokines such as granulocyte macrophage colony-stimulating factor and C-CSF, with or without chemotherapy priming, that mobilized significantly greater quantities of CD34+ cells into the PB, making it possible to collect transplantable doses in a shorter period of time; and (d) myeloid engraftment from PBSC transplant was more rapid and more predictable than that observed with BM, thus reducing the risks of major or fatal infection.

Given average doses of 4.0 × 10⁶ CD34+ cells/kg, myeloid engraftment, defined as a PB PMN level of ≥5 × 10⁹/liter, was observed in 8–10 days for primary refractory myeloma patients. Infusing larger doses of CD34+ cells did not shorten the time to myeloid engraftment, but the rate at which the PMN count rose and the magnitude of the PMN rise was a function of the dose of CD34+ cells given/kg. Rapid engraftment was also observed in patients with breast cancer, Hodgkin’s disease, and lymphoma registered on autologous PBSC protocols, suggesting that different dose regimens did not have to be studied for each individual disease. These principles would subsequently be applied to allogeneic PBSC transplants when they were introduced in the 1990s.

Platelet engraftment was not as uniform or as robust as that of PMN recovery. The platelet level accepted to represent engraftment, 20 × 10⁹/liter, is actually a value that is used to discontinue platelet transfusion support. There was a poor correlation between the dose of CD34+ cells infused/kg and the time to platelet engraftment (21). The majority of CD34+ cells expressed CD33, indicating that they were already committed to myeloid differentiation. Because megakaryocytic precursors represent less than 0.5% of BM cells, the proportion of CD34+ cells that might represent early megakaryocyte progenitors would be expected to be minute compared to the number of myeloid and erythroid progenitors, so that in and of themselves, the dose of CD34+ cells would not be expected to correlate with the time to platelet engraftment. For autologous transplants, it seems intuitively obvious that the level of megakaryocyte precursors might also be inversely correlated with the extent of the patient’s prior chemotherapy.

Regulation of human megakaryopoiesis comprises at least two different steps: (a) a dividing compartment at early stages of differentiation; and (b) a compartment of nondividing cells that increase their size by polyploidization at later stages. The kinetics that transpire in proliferation and endoreplication of megakaryocytic progenitors before platelet production can begin are more complex than the kinetics of myeloid proliferation and have not been clearly defined for in vivo clinical settings. It is likely that multiple factors, including decreased quantities of precursors, slow production rates; clinical factors such as consumption and/or sequestration, disease status, infectious and/or immunological factors contribute to variable platelet recovery.

Clinical protocols that used the abundant PB MNC populations for immune modulation preceded interest in MNCs for transplantation, although protocols using this abundant PB population did not achieve the magnitude of interest achieved with PBSCs. There was a brief but unsustained interest in lymphokine-activated killer cells, but the potential immunological benefit may be realized more fully in the current work on a phenomenon called “graft versus leukemia.” Reports by Slavin et al. (22) in 1988 that CML patients who relapsed cytogenetically or hematologically after allogeneic BM transplant could be reinduced into remission with infusions of DLIs led to protocols to investigate the immunological capacities of PB MNCs. Studies by Giralt et al. (23) using CD8-depleted DLIs have confirmed Slavin’s early data and seem promising.

Present and Future. There are a diverse number of ways in which PBSC support can be used that include: (a) low-dose PBSC support for multiple courses of nonmyeloablative but marrow-suppressive drugs such as Taxol; (b) myeloablative therapy for patients with disease categories not previously offered transplant, such as sarcoma and lung cancer; (c) dual or multiple transplants; and (d) matched unrelated and/or mismatched related allogeneic transplants. Combinations of existing or new cytokines to increase stem cell yields in general or to increase specific targeted lineages such as thrombopoietin for megakaryocytic precursors are likely to be incorporated into transplant protocols as quickly as possible.

Immunotherapy protocols for graft versus leukemia with DLIs are likely to expand to other cells and disorders other than relapsed CML. Certainly there are ample CD4+, CD8+, natural killer cells, dendritic antigen-presenting cells, monocytes, and macrophages in the PB that could be harvested for immunotherapy strategies. Cell dose, cell specificity, infusion schedule, and complications of graft versus host reactions are some of the questions to be resolved by investigators before DLI or other investigative immunotherapies become an established therapies.

Hemapheresis Technology

Hematopoietic supportive care cell therapies would not have been possible without technological tools to implement the perceived clinical needs. In a collaborative effort between the
National Cancer Institute and industry in the 1960s, the first CF-BCS was developed for leukocyte collection (2), but its value for therapeutic leukocyte cytoreduction was quickly appreciated for patients with hyperleukocytosis and leukostasis, described by Freireich et al. (24). In the late 1970s, industry again sought his expertise to develop a second-generation CF-BCS. Originally designed to collect SDPCs (25) and improve PMN collections, protocols for therapeutic platelet cytoreduction and plasma exchange expanded the supportive programs that could be offered to patients. Growth of CF-BCS systems related to the fact that in a centrifugal field, relatively good separation of the various blood components is achieved, based primarily on density differences among the cells and plasma. Such systems allowed access to the entire cellular and plasma contents of the PB for collection, depletion, or exchange. To this end, a series of investigations conducted over a period of 30 years with CF-BCS devices ultimately led to the identification of some common reproducible principles that predicted the outcome ofapheresis procedures reasonably well. These basic characteristics indicated that: (a) the yields of the individual cellular species related most strongly to the quantity of the cells available in the intravascular space to be collected (a product of the PB cell concentration and the BV of the donor/patient); (b) the cellular quantity mobilized from extravascular reservoirs to the intravascular space during the procedure; (c) the fraction of the BV processed to harvest the cells of interest; and (d) the efficiency of the device. The quantity of cells available to be harvested is a biological factor that cannot be predicted, but it can be calculated from preprocedure laboratory data. The magnitude of cell mobilization is also a biological variable that changes dynamically during procedures and that, to date, cannot be predicted reliably. The fraction of BV processed is a procedure variable that can be selected and can range from less than 1 BV for SDPCs to more than 3× the BV for PBSCs. Device efficiency is determined by engineering design characteristics (26, 27).

The value of predicting cellular yields collected on CF-BCS lies in the fact that once the cell dose of a given cell population could be defined, methods were needed by which those doses could be acquired. Mathematical algorithms that integrated these principles were programmable and resulted in an automated third-generation multifunctional CF-BCS device in the mid-1980s (26).

A new generation of technology is emerging to augment studies in supportive care. Technology to sort and/or purify progenitor cells, purge tumor cells, select or deplete selected immune cells, photopheroactivate immune cells and/or malignant cells, and harvest selected subset progenitor populations for ex vivo culture or gene transduction is already in clinical trial.

It is predicted that the use of any cell population of interest for any future supportive care strategy will continue to rely on the dose/response principles described by Freireich, and the technologies developed for those strategies must be characterized and designed to meet those objectives.

References


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