Peripheral Blood Stem Cells: A Novel Source for Allogeneic Transplantation

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ABSTRACT

Cytokine-mobilized peripheral blood stem cells (PBSCs) are increasingly viewed as a promising alternative to bone marrow (BM)-derived stem cells for allografting in patients with hematologic malignancies. Preliminary results seem to indicate several potential advantages of this approach, such as: A) a more “donor-friendly” and possibly safer stem cell collection procedure; B) the procurement of a significantly larger number of progenitor cells (allowing for graft engineering opportunities); C) a faster hematopoietic engraftment including immunologic reconstitution, and D) comparable rates of acute graft-versus-host disease. Although the superiority of this approach over the traditional BM allografting has not been clearly demonstrated thus far in a randomized trial and many open issues remain, experience is accumulating rapidly, and major transplant centers worldwide seem to have endorsed this procedure. The acceptance of the peripheral blood as the primary source of stem cells for hematopoietic reconstitution in the allogeneic setting is likely to have a profound impact in areas such as graft-versus-leukemia/tumor effect, unrelated donor registries, and transplants. In the following, currently available information on blood stem cell harvesting and allografting is reviewed with the particular focus on donor safety.

INTRODUCTION

The last two years have witnessed a dramatic increase in the use of peripheral blood stem cells (PBSCs) in lieu of bone marrow (BM) for allografting in patients with hematologic malignancies in major marrow transplant centers worldwide. A transient shifting of progenitor cells from extravascular sites into the circulation by conventional chemotherapy (chemopriming) and/or cytokine treatment enables the collection by apheresis of a sufficient number of progenitor cells to guarantee engraftment. The administration of recombinant human granulocyte colony-stimulating factor (rhG-CSF) has emerged as an efficient and usually well-tolerated way to accomplish this peripheralization of stem cells, also called mobilization. For the donor, advantages of PBSC collection over traditional BM harvesting include avoidance of anesthesia and surgery, as well as the lack of need for blood transfusions or hospitalization. In terms of clinical outcome, when compared with BM progenitor cells, the use of PBSCs seems to be associated with at least comparable (if not faster) recovery of leukocytes, and faster platelet recovery following transplantation. Allogeneic transplantation of PBSCs does not seem to be associated with a measurable increase in the incidence and severity of acute graft-versus-host disease (GVHD). Due to the more than one log higher number of lymphoid subsets contained in a PBSC allograft, one might expect a faster immunologic recovery and, possibly, a more pronounced graft-versus-leukemia (GVL) effect in the transplant patient. Insufficient data are available currently to address the issue of chronic GVHD. As with BM cells, ex vivo manipulation of mobilized apheresis products is used or being developed (i.e., density-gradient centrifugation, CD34+ cell selection, selection of graft-facilitating cells, purification of CD34+ Thy-1+ lin- progenitor cell subsets, and others) to engineer allografts. It is expected that, based on the easier procurement of hematopoietic stem cells and advantageous engraftment characteristics, PBSCs may in the near future replace, at least in part, BM-derived progenitor cells.
Circulating Hematopoietic Progenitor Cells

BM and PBSC pools are in dynamic equilibrium to each other, allowing hematopoietic progenitor cells migrating from extravascular marrow sites into circulation and vice versa [1]. Both stem cells and their progeny express CD34, a cell surface protein present on 1%-4% of low-density BM mononuclear cells (MNCs) [2]. Greater than 90% of CD34+ cells express antigens that are characteristic of commitment to the lymphoid, myeloid, or erythroid lineages, and, therefore, are not considered stem cells with pluripotent reconstructive potential [2]. On the other hand, CD34+ cells that, for example, lack the CD38 antigen [3] encompassing only 1% of the CD34+ cells [4] are considered pluripotent stem cells with self-renewal capacity.

CD34+ cells and subsets are found in the unperturbed PB from normal PBSC donors in a concentration of approximately 4 × 10⁶/l [4]. For complete and sustained hematopoietic engraftment after myeloablative chemo- or chemo/radiotherapy, patients must receive a sufficient number of early and pluripotent hematopoietic progenitor cells with indefinite self-renewal potential. In humans, the minimum number of early progenitor cells that guarantee complete and permanent engraftment is unknown.

Mobilization of Hematopoietic Progenitor Cells from Extravascular Marrow Sites or Marginal Pools into the Circulation

For obvious ethical reasons, mobilization of PBSCs from normal donors for allogeneic transplantation cannot rely on chemotherapy priming as is the case in autologous PBSC transplantation. Cytokine priming, however, has emerged as an acceptable and efficient treatment alternative for stem cell mobilization. Experience in cancer patients provided the framework for application in normal donors. Systemic treatment with rhG-CSF expands the circulating stem cell pool, exceeding the pretreatment level by approximately 10-fold [5, 6]. Stem cell peripheralization with recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) may be less effective in increasing circulating levels of CD34+ cells [7]. Combinations of interleukin 3 (IL-3) and GM-CSF [8] or G-CSF [9] may have synergistic effects on progenitor cell mobilization. Stem cell factor (c-kit ligand) also mobilizes progenitor cells and has synergistic effects with G-CSF in primates [10] and humans [11].

PBSC Mobilization and Collection in Normal Adult Donors Using rhG-CSF

Advantages of PBSC Collection Over Marrow Harvesting

BM harvesting has now an extensive track record and is considered safe [12]. It remains, however, a surgical procedure and frequently involves general anesthesia and, possibly, a brief hospitalization. The majority of donors also receive or require blood transfusions, which are usually autologous but may occasionally be allogeneic, particularly in the elderly [12, 13]. The morbidity related to the procedure can be substantial in some donors. It occurs mainly in the form of low back pain and/or difficulty walking, may last for up to several weeks (or occasionally months), and therefore may interfere with the donors’ quality of life or affect their return to work [14].

PBSC collection following rhG-CSF mobilization, on the other hand, can be performed in the outpatient setting and does not require anesthesia or surgery. Similarly, autologous (and particularly allogeneic) transfusion is avoided, and the donors can usually resume their usual activities within one to two days of the procedure without significant sequelae [15].

Optimal Cytokine for PBSC Mobilization in Normal Donors

rhG-CSF has emerged as the preferred cytokine for PBSC mobilization in normal donors partly based on the positive experience and toxicity profile reported with its administration to granulocyte donors [16, 17]. rhGM-CSF appears to be less effective in mobilizing CD34+ progenitors in normal stem cell donors, although comparative data are scarce. In a recent study [18], rhGM-CSF was similarly well tolerated in normal individuals and led to a preferential mobilization of early (CD34+ HLA-DR+ CD38+) progenitors among the CD34+ cells. The combination of rhGM-CSF and rhG-CSF did not translate into a higher mobilization of CD34+ progenitors when compared with rhG-CSF alone, but resulted in a significantly greater peripheralization of early CD34+ subsets with the CD34+38− and CD34+ HLA-DR+ CD38− phenotype. These findings suggest that rhGM-CSF deserves further investigation as a mobilizing cytokine in normal individuals.

There is now a significant amount of data which has recently been reviewed on the short-term safety profile of rhG-CSF in normal apheresis donors [19]. The most commonly reported adverse effects, which are partly dose-related, include bone pain, headache, fatigue, and nausea. They ordinarily resolve within a few days of rhG-CSF discontinuation and can be successfully managed in most cases with minor analgesics. Severe adverse events requiring rhG-CSF discontinuation have been rare. rhG-CSF-induced laboratory abnormalities include transient increases (about two- to threefold) of alkaline phosphatase and lactate dehydrogenase, and, less commonly, decreases in serum potassium and magnesium. These are seemingly related to the expanding myeloid cell mass [15].
Continuous-Flow Stem Cell Apheresis

PBSCs are collected by single or multiple continuous-flow apheresis under cytokine treatment. The total blood volume processed per run is between two and three and one-half times the donor’s total blood volume. Large-volume stem cell apheresis processing up to six times the donor’s total blood volume has been reported [20-22]. Typically, 3 × 10^6 MNC cells per kg are collected per run with mononuclear cells encompassing between 60% and 90% of total nucleated cells (TNCs) collected. Anticoagulation is usually performed with acid citrate dextrose-A (ACD-A); calcium replacement is required when using ACD-A alone in large-volume leukapheresis. In normal PBSC donors, it is common practice not to place a central line but rather to use the peripheral vein needle approach to avoid the risk(s) involved with catheter placement. In some cases, however, (approximately 3%-5% at our institution) placement of a central line or narrow harvesting may be required because of inadequate peripheral venous access. Large-volume leukapheresis is known to produce significant platelet depletion, which ordinarily translates into a 30%-50% drop in platelet count after apheresis. This may require up to a week to normalize [23]. Reinfusion of the autologous platelet-rich plasma at the end of the procedure may be performed to minimize this inconvenience [24]. Minor electrolyte imbalances (magnesium, potassium) have also been noted, and they may require electrolyte supplementation.

The CD34+ cell concentration in the donor’s PB is predictive for the yield of CD34+ cells in the apheresis product. Whereas steady-state PB CD34+ cell concentrations in unperturbed hematopoietic condition, being in the range of less than 0.1% of TNC analyzed, are less predictive due to the limited sensitivity of the flow-cytometry technique, the pre-apheresis PB CD34+ cell concentration correlates well with CD34+ cell yield [4]. For example, in normal donors, 40 × 10^6 CD34+ cells per liter PB predict for an apheresis yield of approximately 20 × 10^6 CD34+ cells per liter of donor blood processed. In normal donors under four-day rhG-CSF treatment at 6 µg/kg every 12 hours, the yield is approximately 50 × 10^6 CD34+ cells/kg recipient and per liter of donor blood processed.

Cryopreservation of Apheresis-Derived Stem Cell Products

Apheresis products for autologous transplantation purposes are usually frozen and stored in liquid nitrogen or mechanical freezer below -120°C. The cryoprotectant used is DMSO at a final concentration of between 5% and 10%. Cells are frozen at a controlled freezing rate of 1-2°C/min. Apheresis products from normal donors for allogeneic transplantation are either frozen or transfused freshly. Cryopreserving stem cells has the advantage of being independent from delivering the transplant therapy to the patient. It also has been suggested that there may be a selective loss of alloreactive, GVHD-inducing cells by the cryopreservation and thaw procedures [25].

Effect of rhG-CSF Treatment on the Peripheralization of White Blood Cells (WBC), Polymorphonuclear (PMN) Cells, Lymphocytes, and CD34+ Cells and Subsets in Normal Subjects

To assess the effects of rhG-CSF (12 µg/kg/d) on the peripheralization of hematopoietic progenitor cells and lymphoid subsets, we studied a cohort of 41 normal blood stem cell donors. After three days of rhG-CSF treatment, the WBC, PMN, and lymphocyte concentrations in the donor’s PB exceeded baseline by 6.4, 8.0, and 2.2-fold, respectively [4]. A similar increase of T lymphocytes by day 3 of 16 µg/kg/d rhG-CSF has been reported by Weaver et al. [26], namely 1.5 to 2.0 times over baseline. On the other hand, PB CD34+ cells and primitive subsets such as CD34+ Thy-1^dim, and CD34+ Thy-1^dim 38 cells increased by 16.3-fold, 24.2-fold, and 23.2-fold, respectively, suggesting a selective peripheralization effect of rhG-CSF on hematopoietic progenitor cells and, in particular, on their more primitive stem cell subsets [4].

Kinetics of CD34+ Cells and Subsets Under rhG-CSF Mobilization Treatment

The kinetics of WBC and progenitor cell subsets under rhG-CSF treatment are quite uniform in an unperturbed, normal hematopoietic system, although a remarkable interindividual variability in the degree of progenitor mobilization has become evident [27-29]. When monitored over six days on a daily basis under rhG-CSF treatment (12 µg/kg/d), the kinetics of circulating CD34+ cells and subsets paralleled each other, reaching a plateau from day 4 on (day 1 = first day of cytokine treatment) (Fig. 1). Based on those data and data reported by Tjønnfjord et al. [27] using 10 µg/kg/d rhG-CSF, the most favorable day for stem cell collection would appear to be day 4 or day 5. Continuation of rhG-CSF administration beyond a five-day course leads to a progressive decline in the mobilization of CD34+ progenitors [29, 30].

Duration of rhG-CSF Mobilization and Apheresis Yield of CD34+ Progenitor Cells and Lymphoid Subsets

The duration of rhG-CSF mobilization treatment predicts for the leukapheresis yield of CD34+ progenitor cells. We studied 77 normal donors who underwent stem cell apheresis for HLA-matched, related recipients beginning on day 4 (n = 45) or day 5 (n = 32) of rhG-CSF treatment (12 µg/kg/d). Both cohorts were comparable for age, weight, and blood volume processed by apheresis, and target CD34+ cell dose of 4 × 10^6/kg of recipient body weight to be collected. The
day 5 schedule allowed a more consistent achievement of target cell dose with one apheresis, and resulted in the initial collection of a significantly larger number of CD34+ cells. There was no statistically significant difference in the apheresis yield of lymphoid subsets or natural killer cells, although a trend toward a higher CD3+ lymphocyte yield was found [31].

Dose-Dependent Mobilization of CD34+ Progenitor Cells

It has been shown that, at least for rhG-CSF doses up to 10 µg/kg/d, a dose-response relationship exists between rhG-CSF dose and degree of mobilization of CD34+ progenitor cells [30, 32]. Although rhG-CSF doses up to 24 µg/kg/d have been employed [33, 34], experience with these doses remains limited, and it is unclear whether they will prove to be necessary or cost-effective to achieve the collection of an “adequate” alloengrafting CD34+ cell number.

Factors Affecting Mobilization of CD34+ Progenitor Cells

In an effort to elucidate factors affecting mobilization in normal donors and stem cell yield by apheresis, the CD34+ cell yield from the first day of apheresis in 119 donors who underwent apheresis on days 4-6 of rhG-CSF treatment (12 µg/kg/d) was analyzed [35]. The CD34+ cell yield was significantly lower in donors >55 years of age, or who were not obese. There was also a weak correlation between CD34+ cell yield and age, baseline WBC count, pre-apheresis WBC count, and pre-apheresis MNC count. Twenty-one (18%) donors were considered “poor mobilizers,” yielding fewer than 20 × 10^6 CD34+ cells/l blood processed. In the multivariate analysis, the only significant risk factor for inferior mobilization was age >55 years, which conferred a 3.8-fold increased risk (p = 0.04). As poor mobilizers occurred in all age groups, however, the predictive value (and clinical usefulness) of the model was limited.

Transient Post-Donation Cytopenias in Normal Donors

As discussed previously, large-volume leukapheresis has been reported to cause a decrease in platelet count [20, 23]. A direct role of rhG-CSF has also been shown in this setting [36]. More recently, asymptomatic, transient drops in lymphocyte and granulocyte counts below baseline levels occasionally resulting in lymphopenia or granulocytopenia have been reported following rhG-CSF mobilization and stem cell collection [36-39]. The impact on different lymphocyte subsets, which can last for several weeks after collection, has also been evaluated [38, 39]. The relevance (if any) of these findings is presently under investigation.

From the data reported so far, one can assume that the circulating progenitor cell pool has regained a stable, steady-state condition at around 30 to 100 days post blood stem cell collection. Second stem cell collections in normal apheresis donors have been performed (even as early as one to two months after the first), and seem to provide comparable yields of CD34+ progenitor cells [40]. These data support the concept that mobilization efficiency in normal donors remains relatively unchanged over time and is not significantly altered by rhG-CSF exposure.

PBSC MOBILIZATION IN Pediatric DONORS

Given the promising data reported with clinical trials on allogeneic PBSC transplantation in adults and the potential advantage to the donor over a BM harvest under general anesthesia, collection of PBSCs in normal children is an appealing approach. In the autologous transplant setting, rhG-CSF treatment at a dose ranging from 5 to 10 µg/kg/d, combined with chemopriming for transient stem cell peripheralization and collection, is well tolerated in small children [41, 42]. As compared to adverse effects of rhG-CSF mobilization treatment reported by us in normal adult stem cell donors [43], normal pediatric donors seem to tolerate rhG-CSF treatment up to 12 µg/kg/d as well.

Venous access in small children providing sufficient blood flow is the major limiting factor for apheresis. To ensure an adequate and consistent blood flow rate of 20 ml/min and more during apheresis, Takaue et al. [42] successfully used a
temporary radial artery catheter for blood withdrawal and return through a peripheral vein.

In a series of five allogeneic blood stem cell transplants using pediatric donors between 4 and 13 years, we processed a median of 2.2 times the donor’s total blood volume (TBV) per apheresis, which provided a sufficient CD34+ cell yield per kg of donor body weight and per liter of donor blood processed was $128 \times 10^6$ in these five children [43] as compared to $63 \times 10^6$ for adult donors [4]. As shown in an eight-year-old boy, a single apheresis under rhG-CSF mobilization treatment was sufficient to collect a CD34+ allograft dose of $4 \times 10^6$ for his father diagnosed with chronic myelogenous leukemia (CML), despite the substantial body weight discrepancy between the pediatric donor (27 kg) and adult recipient (93 kg) [43].

Pediatric blood stem cell collection from normal donors is feasible and safe with adequate cell doses, even for adult recipients. Engraftment characteristics and clinical outcome appear comparable thus far to the ones achieved with adult PBSC allotransplantation.

**PROGENITOR CELL DOSE REQUIREMENTS FOR SUCCESSFUL ALLOGRAFTMENT AFTER MYELOABLATIVE TREATMENT**

The indicators for hematopoietic progenitor cells with reconstitutive potential contained in the apheresis products are short-term/long-term culture assays or, more recently, the CD34 surface antigen. Since clonogenic assays are time-consuming and difficult to standardize, the immunophenotyping of progenitor cells has widely replaced the colony-forming unit assay. The minimal CD34+ cell dose required in the autologous transplant situation for complete three-lineage engraftment is not well defined, but may be in the range of $1 \times 10^6$ for adults [44]. There is more consistent rapid recovery with higher CD34+ cell doses, and a level between 2 and $5 \times 10^6$ has been recommended as the safe engrafting dose by different centers. For complete and sustained allogeneic engraftment among HLA-matched siblings, we propose the transfusion of $>3-4 \times 10^6$ CD34+ cells per kg of recipient body weight. At our institution this target dose is reached with one leukapheresis in approximately 80% of the donors and with two in the majority of the others [45]. A similar target dose ($>2-3 \times 10^6$ CD34+ cells per kg) has recently been recommended by the European Group for Blood and Marrow Transplantation (EBMT) [46].

**ALLOGENEIC PBSC TRANSPLANTATION: ENGRAFTMENT CHARACTERISTICS**

The literature which followed the initial reports on allogeneic PBSC transplantation was primarily focused on engraftment characteristics [47-49]. Unfortunately, a comparative analysis of these reports is made difficult by the small sample size, significant differences in terms of patient characteristics, conditioning regimens, supportive care, and GVHD prophylaxis (with or without methotrexate), as well as the non-randomized nature of these studies. Nevertheless, some general conclusions can be drawn from these data. When compared with historical control patients receiving marrow allografts, the recovery of granulocytes and platelets seems to be at least comparable and, according to most investigators, faster. This seems to be true particularly for platelet recovery [49-51]. In view of the fast myeloid engraftment after allogeneic PBSC transplantation, it is unclear whether post-infusion cytokines (i.e., rhG-CSF) are actually beneficial or cost-effective [49].

**ALLOGENEIC PBSC TRANSPLANTATION: TREATMENT-RELATED TOXICITY**

In the field of allogeneic transplantation, treatment-related toxicity is usually thought to include regimen-related organ toxicity (largely dependent on the conditioning regimen), infectious and bleeding complications (requiring antibiotics/antifungal agents as well as platelet transfusions), and GVHD (acute and/or chronic). The impact of PBSC allografting on regimen-related toxicity and infectious or bleeding complications has not been fully evaluated. We analyzed early treatment-related morbidity and mortality after HLA-matched allogeneic transplantation of rhG-CSF mobilized PBSC as compared to BM allografts [50]. Three cohorts of patients were analyzed: cohort I ($n = 30$) received a BM allograft and was treated with cyclosporine-A + methotrexate for GVHD prophylaxis; cohort II ($n = 19$) received a BM allograft as well, but was prophylactically treated with cyclosporine-A + methylprednisolone, and cohort III ($n = 25$) received a PBSC allograft with the same GVHD prophylaxis as cohort II. rhG-CSF (5 µg/kg/d) was given to all patients after transplant to enhance hematopoietic reconstitution. There was less regimen-related toxicity (especially stomatitis) in PBSC transplant recipients, and this was not accounted for by a shorter duration of neutropenia. PBSC recipients (cohort III) were discharged from the hospital on the average four days earlier than their BM counterparts (cohort II). The 180-day survival was significantly higher in the allogeneic PBSC transplant group (cohort III) with 68%, as compared to 53% in cohort I and 32% in cohort II. Azevedo et al. have described significantly shorter hospital stays as well as fewer days on antibiotic and antifungal agents in PBSC recipients [52]. Russell et al. have reported shorter hospitalization stays and fewer platelet transfusions for PBSC allograft recipients as compared to BM recipients [51].

Whereas the average number of CD34+ cells collected is four times higher than with a BM allograft, the number of
T and natural killer (NK) cells in the apheresis product exceeds the BM allograft by 10- to 20-fold [4], leading to concern that PBSC allotransplants could produce more severe GVHD. The great majority of available data suggests that the incidence and severity of acute GVHD following PBSC allografting are similar to (or possibly even lower than) that encountered after marrow allografting [33, 47-50, 52-56]. More direct comparisons, particularly the ones employing the same GVHD prophylaxis regimens, are in keeping with these findings [50, 52], although they have employed historical or nonrandomized controls. It is possible that development of acute GVHD requires a critical threshold of lymphoid cells, and once this threshold is reached the infusion of a higher number of lymphocytes does not necessarily translate into more frequent or severe acute GVHD, although other mechanisms may be responsible for this finding.

The issue of chronic GVHD following PBSC allografting has to be considered open, however, due to the small sample size and the short follow-up in essentially all of the studies mentioned above. It is well recognized that the addition of buffy coat cells to marrow infusion leads to a higher incidence of chronic GVHD [57]. We have studied a group of 47 consecutive allogeneic PBSC transplant recipients surviving at least 100 days after transplant, and observed a significantly higher rate of (mainly extensive) chronic GVHD following allografting with PBSCs than with BM [58]. This was confirmed at our most recent data analysis (median follow-up 10 months), although it did not translate into a higher mortality because of a lower incidence of relapse in PBSC recipients. This lower relapse rate (if confirmed by other investigators) would be consistent with an enhanced GVL effect [58]. Should chronic GVHD turn out to be a significant problem after PBSC allografting, ex vivo T cell depletion of the apheresis product or a positive selection of CD34+ cells may be considered [24, 59].

**Immunologic Reconstitution after Allogeneic PBSC Transplantation**

As stated above, the number of T and NK cells in the apheresis product exceeds the BM allograft by 10- to 20-fold [4]. Apart from GVHD, this would also be expected to impact the speed of immunologic reconstitution after PBSC allografting. Ottinger et al. [60] most recently reported an improved immune reconstitution after allogeneic PBSC transplantation as compared to BM allotransplantation. Naive (CD4+ CD45RA+) and memory (CD4+ CD45RO+) helper T cells were found to be significantly elevated in patients receiving an allogeneic PBSC transplant, and proliferative responses to phytohemagglutinin, pokeweed mitogen, tetanus toxoid, and candida were found to be more pronounced as well.

In another report [47], the recovery of CD3+ cells was similar in allogeneic PBSC recipients and concurrent marrow transplant recipients. On the other hand, the recovery of CD4+ and, to a lesser extent, CD8+ cells was significantly faster in the former group. This translated into a more rapid increase in the CD4/CD8 ratio as well. Whether this more rapid immunologic reconstitution will translate into decreased morbidity and mortality from infectious complications (e.g., cytomegalovirus, fungal infections) or, for that matter, enhanced GVL activity, remains to be determined.

**Ex Vivo Manipulation of the Allogeneic PBSC Graft**

Most of the reported studies on allogeneic PBSC transplantation have relied on unmanipulated apheresis products. Only recently, data on manipulation of the apheresis product prior to PBSC allografting have been reported. The larger number of CD34+ cells collected by PBSC apheresis [4] allows for some selective loss of progenitors during these maneuvers. Link et al. have described allogeneic transplantation employing positively selected CD34+ PBSCs, accomplishing at the same time a 100- to 1,000-fold reduction in the number of T cells [24]. Engraftment was rapid. Despite GVHD prophylaxis with cyclosporine, severe acute GVHD (grade III-IV) developed in four of the first five patients. Similar data have been reported by the Seattle group [61]. Employing a similar approach, however, Urbano-Ispizua et al. obtained rapid engraftment with virtual elimination of grade II-IV acute GVHD [62]. An alternative approach has been to perform a density-gradient centrifugation to achieve CD34+ enrichment, and cell “debunking” of apheresis products [59]. Although graft engineering is appealing and may eventually allow optimization of the allograft characteristics, the field is currently in its infancy.

**Outstanding Issues Regarding Transplantation of PBSCs**

Although data are accumulating rapidly, the details of the stem cell collection procedure should be addressed in order to maximize the stem cell yield and, at the same time, minimize donors’ discomfort and exposure to rhG-CSF. Randomized trials are needed to optimize collection conditions and define the preferred rhG-CSF mobilization dose and PBSC dose for allografting. If available data suggest that the short-term safety profile of rhG-CSF in normal apheresis donors is acceptable [19], the issue of possible long-term effects (i.e., the development of myelodysplasia or myeloid leukemia) remains open and difficult to address. Available data are largely limited to isolated case reports [63]. It has been estimated, for instance, that to detect a 10-fold increase in the leukemia risk (a substantial risk increase), more than
2,000 normal donors would need to be followed for up to 10 years or longer, and the detection of a smaller-risk increase would require the follow-up of a comparably larger donor pool [64]. This can conceivably be accomplished only by international registries and will probably require an intensive cooperative effort with individual transplant teams and centers. The data available on the long-term effects of rhG-CSF in patients with severe congenital neutropenia and aplastic anemia do not answer these questions, as these diseases have been shown to carry a predisposition to the development of acute leukemia regardless of rhG-CSF therapy [65, 66].

Partly related to the long-term effects issue is the issue of PBSC collections in unrelated, HLA-matched donors. Only a very limited number of PBSC allografts from matched, unrelated donors have been performed worldwide to date [46]. Until now, in the United States the National Marrow Donor Program (NMDP) has endorsed PBSC collections only for second transplants (i.e., for treatment of relapse following marrow transplantation). Currently, many national registries are gradually becoming more open to the idea of administering rhG-CSF to unrelated HLA-matched donors, although some logistical issues and safety concerns still remain. PBSC collection seems to be gaining increasing acceptance in the blood banking community as well [67]. A more “user-friendly” and possibly safer collection procedure may allow a substantial expansion of the unrelated stem cell donor pool in national and international registries, particularly among minorities and older individuals [68].

Finally (and most importantly), before the PBSC collection procedure can be endorsed and can replace marrow for allografting, measurable benefits over marrow transplantation in terms of patient outcome parameters will need to be demonstrated. These parameters should include variables such as patient transfusion requirement and duration of hospitalization, reduction of treatment-related mortality, and improvement of survival and/or disease-free survival. Preliminary data now available look promising, but additional work is clearly needed. A large randomized trial sponsored by the EBMT [46] is currently in progress and will hopefully clarify some of these issues.

**CONCLUSIONS**

Table 1 summarizes the advantages and disadvantages of the PB as compared with BM as a source of hematopoietic progenitor cells for allogeneic transplantation. The use of PBSCs for transplantation represents a major advance. The temporary peripheralization of hematopoietic progenitor cells allows collection of large doses of progenitors and has a significant advantage for the donor, eliminating the need for general anesthesia and multiple bone marrow aspiration. Allogeneic PBSC transplantation provides rapid hematologic recovery, and it may reduce hospitalization and costs. The optimal dose of cells, means of mobilization, and cellular composition of the stem cell graft need to be further investigated.

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<th>Table 1. Advantages and disadvantages of peripheral blood versus bone marrow as a source of hematopoietic progenitor cells for allogeneic transplantation</th>
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<td><strong>Peripheral blood</strong></td>
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<td><strong>Advantages in collection and allogeneic transplantation</strong></td>
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<td>• Larger number of CD34+ cells</td>
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and finely tuned. Allogeneic PBSC transplants do not appear to increase the risk of acute GVHD in preliminary studies, but long-term study of their beneficial effects is required. Overall, the PB appears at least as effective as BM as a source of hematopoietic stem cells for allogeneic transplantation. The ease and safety of collection as well as initial data with PBSC allotransplants provide justification for further evaluation. In the long term, it appears likely the PBSCs will replace BM, at least in part, as the preferred source of hematopoietic cells for transplantation.

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