Peripheral blood stem cell transplantation ; an update

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Abstract : Patients with a number of different malignancies have been treated with high-dose chemotherapy and peripheral blood stem cell transplantation (PBSCT). PBSC already replaced bone marrow as the source of autologous hematopoietic progenitor support. This is due to ease of collection, rapid engraftment and less possibility of tumor cell contamination in the graft. Furthermore, allogeneic transplantation of granulocyte colony-stimulating factor (G-CSF) mobilized PBSC is now being increasingly performed. Recent advance of clinical PBSCT and new strategies are stressed in this review. New strategies include CD 34+cell purification, *ex vivo* expansion of PBSC and PBSC as a target cell for gene therapy. Major future advance may occur better understanding of the mechanism of mobilization and the biology of PBSC. J. Med. Invest. 44 : 25-31, 1997

Key Words : *peripheral blood stem cell transplantation, mobilization, autologous, allogeneic, CD34⁺cell*

INTRODUCTION

The field of stem cell transplantation continues to advance very rapidly (1). The term "*bone marrow transplantation*" now has become less used. Instead of bone marrow cells, peripheral blood stem cells (PBSC), or cord blood cells are increasingly used for hematopoietic rescue operations. In the 1980 s, we recognized that hematopoietic progenitor cells circulated in the blood during the marrow recovery phase of myelosuppressive chemotherapy and could be concentrated for transplantation by repeated aphereses (2, 3). Thereafter hematopoietic growth factors came into clinical use, which markedly improved the efficiency of PBSC collection and accelerated hematopoietic recovery following transplantation (4). Over the last decade, the use of PBSC and better supportive care techniques have made high-dose chemotherapy comparatively safe with less than a 2-3% mortality rate in experienced centers (5). Thus, it has now been widely applied even in community hospitals (6). Recently, PBSC also began to be applied as an allogeneic stem cell source for the treatment of hematologic malignancies (7). Nonetheless, the exact mechanism of stem cell mobilization is poorly understood, and the biology of PBSC remains incompletely defined. In this review, we update recent progress in this field.

PERIPHERAL BLOOD AS A STEM CELL SOURCE

Mobilization

There is little question against the need to mobilize progenitor/stem cells for practical collection. Mobilization of progenitor cells from the bone marrow (BM) to the PB can be achieved with myelosuppressive chemotherapy alone, or in combination with hematopoietic growth factors, or with hematopoietic growth factor alone administered in an hematologic steady state. Myelosuppressive chemotherapy was the first measure used for mobilization (8). In this way the timing of apheresis is rather unpredictable and toxicities such as neutropenic fever are inevitable (9). With the advent of growth factors, myelosuppressive chemotherapy alone is less used for mobilization. Mobilization methods by growth factors alone are certainly more convenient since collection days can be more precisely timed. Granulocyte colony-stimulating factor (G-CSF) or granulocyte/macrophage colony-stimulating factor (GM-CSF) alone enhance mobilization in both cancer patients and normal healthy donors (10, 11). In the allogeneic setting, G-CSF is exclusively used because its administration is safer and less toxic compared with other available cytokines including GM-CSF (12). Other cytokines or combinations of current or new growth factors, i.e., stem cell factor, interleukin-3 and thrombopoietin, are being studied as more efficient mobilizers (13). However, additional advantages of these factors to G-CSF are still unknown.

The exact mechanism by which progenitor cells are mobilized is unknown. Evidence is now emerging that cell adhesion molecules (CAMs) may be involved in the process of mobilization (14, 15). We studied several CAM expressions on PB and BM CD 34⁺ cells and found a significantly lower expression of integrins such as very late antigen-4 (VLA-4), leukocyte function antigen-1 (LFA-1) and LFA-3, and a significantly higher expression of L-selectin and CD44 on PB CD34⁺ cells compared with BM CD34⁺ cells. This suggests that decreased expression of CAMs play a role in progenitor cell mobilization. Progenitor cell mobilization might occur as a stochastic process and involve the selection of CD34⁺ cell with low

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CAMs. Some role might be attributed to a loss of function of integrins, and the disruption of VLA-4 ligand receptor binding results in the release of progenitor cells in primates (16).

Collection and storage

Cell separators widely used for PBSC collections are Spectra (Cobe Laboratories), CS 3000 (Baxter Health Care Corp.) and AS 104 (Fresenius AG). Harvesting PBSC is safe and does not involve the risk of general anesthesia (17) or multiple invasive marrow aspirations. Adverse effects related to apheresis procedures occurred in less than 3% of adults (18). These included moderate hypovolemia and symptomatic hypocalcemia with perioral paresthesia which usually promptly resolved with infusion of calcium gluconate.

For cryopreservation of PBSC, a simple freezing method using 6% hydroxyethyl starch (HES), 5% dimethyl sulfoxide (DMSO) and 4% albumin has been used (19). This procedure is simple and requires less processing time compared with the conventional method which employs controlled-rate freezing by a programmed freezer and use of 10% DMSO (20). For storage of PBSC, a-135℃ mechanical freezer replaced the storage in liquid nitrogen at some transplant centers. PBSC isolated from apheresis products can be stored in a-80℃ mechanical freezer, but the duration of frozen storage should not exceed 1.5 years (21).

Monitoring PBSC collection efficiency

Colony forming units-granulocyte/macrophage (CFU-GM) and $CD34^+$ cells are the two widely accepted indicators of the hematopoietic reconstitutive capacity of transplanted cells. The dose-effect relationship between the CFU-GM content of the graft and the speed of recovery following PBSCT has been shown in several studies(22). Standardization of CFU-GM measurements remains elusive due to the variations in methodology used in different laboratories. The measurement of $CD34^+$ cells using a flow cytometer is much quicker than CFU-GM assays, which require at least 2 weeks to obtain the final results (23). However, $CD34^+$ cell enumeration needs the level of stringency to measure rare event at the level of 0.1% to 3% (9). Issues in achieving standardization in $CD34^+$ cell enumeration include gating strategy, CD34 antibody and degree of cell debris (24).

Characterization of PBSC

The phenotypic analyses of mobilized $CD34^+$ cells are also extensively studied. The most striking differences observed regarding phenotypic profiles of mobilized $CD34^+$ cells were the very high percentage of $CD34^+$ cells coexpressing CD33 and CD13 molecules and the low percentage coexpressing CD10 and CD19 compared with BM $CD34^+$ cells (25). Furthermore, PB $CD34^+$ cells express less c-kit and CD71, suggesting that the mobilization of progenitor cells involves the down-regulation of c-kit (8). Mobilized PBSC showed a low percentage of circulating cells in the S phase despite growth factor administration. This may be due to a selective release from BM cells in the G_0-G_1 phase and/or to the expression of critical cell cycle-related adhesion receptors.

AUTOLOGOUS TRANSPLANT

Initially, PBSCT was used for patients who were not eligible for BM harvest because of tumor involvement, extensive marrow fibrosis following local irradiation or contraindication to general anesthesia (1). Although there was a concern regarding long-term hematopoiesis following PBSCT, recent clinical data supported the durability of normal hematopoiesis after autografting with PBSC. Furthermore, proof of long-term engraftment following infusion of PBSC was obtained by gene-marking studies (26, 27). Since 1990, the number of PBSCT has far exceeded that of BMT in the autologous setting, and nowadays, PBSC have been used in 90% of autologous transplants (28). The major advantage of using PBSC over BM cells is the rapid hematopoietic reconstitution, which results in a shorter duration of antibiotic use, fewer blood product transfusions and early hospital discharge. Although autologous PBSCT should not by themselves lead to a different tumor control outcome than autologous BMT. Various analyses show no advantage of PBSCT over BMT in terms of overall survival rate [Table 1] (29-34). However, economic assessments of PBSCT have proven a direct cost saving effect associated with a reduction in the duration of hospitalization compared with BMT (35, 36). In some cases, PBSCT are performed largely on an outpatient basis to save admission costs (37). In addition, the collection process for PBSC is less invasive than BM harvest, and the donor can avoid general anesthesia. PBSCs may be harvested in an outpatient setting. These properties may increase the pool of unrelated donors in allogeneic transplants.

Indications for autologous PBSCT have changed with time and the types of illnesses treated with this approach continue to increase, within a range of chemosensitive tumors. High-dose therapy with PBSCT has become an established therapeutic option for patients with relapsed, but still chemotherapy-sensitive aggressive non-Hodgkin's lymphomas (NHL) (5). PBSCT has also been shown to produce long-term disease-free survival in selected patients with refractory and advanced Hodgkin's disease (38). Intensified treatment with high-dose melphalan supported by PBSC as a means of overcoming drug resistance yields complete remission even in patients with high-risk and advanced multiple myeloma (39). Yet, earlier transplantation is recommended before the development of drug resistance and end organ damage. Breast cancer has now become the disease most frequently treated with autologous PBSCT worldwide (40). Leading indications moved from refractory or metastatic breast cancer to high-risk or locally advanced breast cancer as an adjuvant consolidative therapy. Germ cell tumors are also a good indication for autologous PBSCT. Although autologous PBSCT also has been performed for childhood cancers such as neuroblastoma,

rabdomyosarcoma and Wilms' tumor, the number of cases is still small and its role for these indications remains to be determined. For acute leukemias, many trials involving autologous BMT or PBSCT have been performed in patients with AML.

Dose intensification and sequential use of agents to overcome drug resistance may benefit some patients, and interest in the concept of tandem transplants has been growing. The principal objective of this procedure is to reduce the size of a slowly growing tumor mass by repeated, closely timed courses of high-dose chemotherapy, each given with hematopoietic stem cell rescue. Tandem cycles of high-dose chemotherapy became feasible with autologous PBSCT, since abundant PBSC yields obtained by apheresis provides the opportunity to deliver more than one cycle of high-dose chemotherapy. These procedures are under intense investigation in the treatment of breast cancer, childhood cancers and multiple myeloma (41-43).

ALLOGENEIC TRANSPLANT

G-CSF mobilized allogeneic PBSC have been used without significantly increased severe GVHD while reproducing the rapid reconstitution seen in the autologous setting (44). A number of recent studies indicated that transplants using allogeneic PBSC had an obvious advantage for accelerated engraftment over conventional BMT (45). Although there was concern that a relatively high number of infused lymphocytes might result in unacceptably severe acute GVHD, this has not been observed in fully matched sibling pairs (46). The early survival rates were similar between allogeneic PBSCT and BMT [Table 1] (44,45). Nevertheless, there is a possibility that the risk of chronic GVHD may increase in allogeneic PBSCT (47).

Regarding stem-cell mobilization in normal donors, two clinical variables should be taken into consideration. The first is efficacy, defined as the ability to mobilize sufficient numbers of PBSC, and the second is toxicity, defined as the side effects that the donor may experience. Although

Table 1. Comparative studies of PBSCT vs BMT

Ref.	Type of transplant	Disease	Investigator /Type of study	Timing	Overall survival (PBSCT vs BMT)	Advantages of PBSCT over BMT
29	Auto	Follicular NHL (n=60)	Bastion, et al./ Comparison with published data	Variable Mostly PR	86% (2 yr.) vs 50% (5 yr.)	Low treatment-related death rate
30	Auto	HD & NHL (n=27 vs 31)	Schmitz N, et al. /Prospective randomized	Variable	87.1% vs 88.9% p=N. S. (median follow-up ; 311 days)	Rapid hematopoietic reconstitution Early discharge from hospital
31	Auto	HD (n=227 vs 227)	EGBMT/ Randomized	Variable	52.7% vs 65.3% p=.0198 (4 yrs)	Rapid hematopoietic reconstitution
31	Auto	NHL (n=128 vs 128)	EGBMT/ Randomized	Variable	52.7% vs 56.6% p=.4148 (4 yrs)	Rapid hematopoietic reconstitution
32	Auto	Adult ALL (n=12 vs 38)	Powles R, et al./ Non-randomized	First CR	11/12 vs 21/38 (median follow-up ; 40 months)	Decreased transplant-related toxicity
33	Auto	Multiple myeloma (n=43 vs 43)	Harousseau JL, et al./ Randomized	First remission induction	45% vs 45% p=0.37 (4 yrs)	Neutrophil recovery
34	Auto	Germ cell tumor (n=23 vs 24)	Beyer J, et al./ Randomized	Relapsed or refractory	41.7% vs 52.2% p=0.39	Rapid hematopoietic reconstitution
45	Allo	AML, ALL, CML, CLL, MDS, MPD, NHL, MM (n=22 vs 21)	Pavletic ZS, et al./ Prospective randomized	Variable	83% vs 75% (100 day) p=0.358	Faster engraftment, Shorter hospital stay
44	Allo	AML, NHL, ALL, HD, CML, MM (n=37 vs 37)	Bensinger WI, et al./ Retrospective comparison	Relapse or >CR 2	50% vs 41% (estimated at 285 days) p=0.39	Faster engraftment, Fewer transfusions, No greater incidence of acute or chronic GVHD

Ref. : References, NHL : Non-Hodgkin's lymphoma, HD : Hodgkin's disease, AML : acute myelogeneous leukemia, ALL : acute lymphocytic leukemia, CML : chronic myelogenous leukemia, CLL : chronic lymphocytic leukemia, MDS : myelodysplastic syndrome, MPD : myeloproliferative disorder, MM : multiple myeloma, EGBMT : the European Group for Blood and Marrow Transplantation, CR : complete remission, PR : partial remission, N.S. : not significant

mobilization with G-CSF thus far seems a safe procedure for normal donors, most donors still complain of general fatigue and myalgias/arthralgias which are relieved easily by analgesia. The platelet count decreased in most donors. Slight but significant decrease in platelet count is observed at the peak of the G-CSF induced leukocytosis. It is suggested that G-CSF administration reduced the capacity for platelet production (48). Furthermore, a decrease in platelet count was subsequently enhanced by an apheresis-related decrease. Although there has been no clinical report of life threatening bleeding, all donors should be carefully monitored. Another concern regarding donor safety is the potential long-term toxicities of G-CSF, which have to be determined with longer follow-up. The theoretical risk of developing leukemia and myelodysplastic syndrome (MDS) after G-CSF treatment may be of concern, but to date, there were insufficient cohorts to evaluate this possible risk (49). The issues still to be determined include more detailed knowledge of G-CSF dosage, optimal PBSC numbers, the dynamics and durability of engraftment and immunologic reconstitution of the recipient (50). The superiority of PBSCT over BMT, particularly from mismatched donors is unproven.

Nowadays, indications for allogeneic PBSCT include high-risk hematological malignancies such as acute leukemias, MDS and chronic myelogeneous leukemia. Non-malignant hematologic disease such as severe aplastic anemias and congenital immunodeficiencies may also be good indications for allogeneic PBSCT.

NEW STRATEGIES

CD34 antigen is expressed on both hematopoietic progenitors and stem cells. The number of progenitor cells expressing CD34 in PB is known to increase following mobilization procedures. $CD34^+$ cell purification technology continues to be improved to obtain cells in greater than 90% purity and greater than 3-log tumor-cell or T-cell depletion (51). Purposes of $CD34^+$ cell purification are to purge tumor cells in the grafts for autologous PBSCT (52) and to deplete T-cells which contribute to the development of GVHD in allogeneic PBSCT (53). In addition, selected $CD34^+$ cells have the advantage of reducing graft volume, facilitating storage and decreasing the amount of DMSO as well as cell lysis products. However, in the autologous setting clonogenic tumor cells are still present in $CD34^+$ cell selected grafts, and their association with relapse is unknown. Recent advances in gene marking studies may provide much-needed information on malignant contamination in $CD34^+$ selected grafts. Isolex 300 is now available for clinical use, although the cost of this equipment for processing exceeds one million yen. The cost-effectiveness should be evaluated carefully. Apheresis products undergoing selection of $CD34^+$ cells have a greater yield and enrichment of progenitor cells compared with BM harvests collected from HLA-identical normal healthy donors (54).

Engraftment with $CD34^+$ purified PBSC confirms that autologous $CD34^+$ cells, alone, are sufficient to provide hematopoietic rescue for myeloablated patients (55). Blood cell recovery following $CD34^+$ cell transplantation is as rapid as after unmanipulated PBSCT, suggesting that there are no adverse effects to removing accessory cells, such as activated monocytes or lymphocytes, on blood cell recovery speed. There is some hope that most patients can have donors if transplants with allogeneic $CD34^+$ cells obtained from haploidentical related donors are performed without deterioration of engraftment and GVHD. However, there is a concern that major risks of an allogeneic transplant with $CD34^+$ cells include increased graft rejection, loss of GVL effect and development of serious viral infection and lymphoproliferative disorders due to delayed recovery of immune function (56). In this regard, add-back of T-cells into patients at an appropriate time following transplant might be a reasonable strategy.

There is considerable interest in the possibility of expanding stem cell *ex vivo*. *Ex vivo* expansion of progenitor cells is a new approach to abrogating cytopenia posttransplant or expanding a small aliquot of mobilized PBSC to provide sufficient progenitor cells for hematopoietic rescue. The specific combination of hematopoietic growth factors and the culture system and/or hematopoietic stroma were identified as important variables for *ex vivo* expansion of PBSC (57). However, the feasibility of this approach in a clinical setting still remains unclear in the lack of an *in vitro* assay for human stem cells. In addition, whether endogenous tumor cells from patients are concomitantly expanded in culture should be monitored carefully in the autologous transplant setting (58).

Clinical application of gene therapy using PBSC in patients with cancer or congenital metabolic diseases has been studied. An advantage of PBSC is that multiple collection procedures can be performed without invasive surgery ; which may be an important consideration in gene therapy (59). The purification of $CD34^+$ cells is again necessary to improve the transduction and/or long-term expression.

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